ADVANCES IN INTERNAL MEDICINE

VOLUME IV

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CONTENTS

Nitrogen Mustards in the Treatment of Neoplastic Disease*

DAVID A. KARNOFSKY
The Sloan Kettering Institute for Cancer Research, and Memorial Hospital, New York

The treatment of inoperable neoplasms is a difficult and often disheartening task The discovery of an agent possessing some degree of therapeutic activity in these conditions, therefore, is an important and welcome contribution The nitrogen mustards have been under intensive clinical investigation since May, 1946. They have not proved of curative value but, in acquiring a useful place in the palliation of some forms of neoplastic disease, they are of interest to all physicians In this review, we propose to describe the history and properties of the nitrogen mustards, their effects on normal and neoplastic tissues, and to define the indications for their use, particularly in relation to other forms of therapy, for the palliative treatment of cancer in man.

Background

HISTORY

The nitrogen mustards derive their name from mustard gas, to which they are closely related chemically and pharmacologically Their history begins and remains intimately connected with that of mustard gas †

Mustard Gas In 1860, Guthrie (97) and Niemann (170) first synthesized bis(2-chloroethyl) sulfide (mustard gas) and found that it was vesicant and injurious to the eyes Meyer (162), in 1886, pre-

* This work was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research

† I wish to express my appreciation to Dr Homer W Smith for permitting me to use his review of the literature on the systemic action of mustard gas prepared for the Office of Scientific Research and Development in 1943

pared pure mustard gas (actually a liquid at ordinary temperature) and demonstrated by animal experiments its necrotizing effects on the skin, respiratory tract and eyes (152). During the next 25 years only a single article on mustard gas, which confirmed Meyer's observations, appeared in the literature (55).

At Ypres, on July 12–13, 1917, mustard gas suddenly gained world prominence when the Germans began to use it as a military weapon It soon became evident that mustard gas was the most effective chemical warfare agent then available, and it quite properly acquired the title, "The King of the War Gases" (180). The most important toxicologic effect of mustard gas was the contact injury it produced, either as a liquid or vapor, to the eyes, skin, and respiratory tract Since these lesions were apparent and by far the more common ones, they were studied intensively. Rarer and more subtle were the systemic effects occurring in some men exposed to large amounts of mustard gas, effects presumably due to the absorption of mustard from the skin, or respiratory or gastrointestinal tracts. The systemic effects to be attributed to mustard had to be differentiated from those produced by extensive vesication, sloughing and infection of the skin, by edema and necrosis in the respiratory passages, by bronchopneumonia, by erosions of the gastrointestinal tract due to the ingestion of mustard gas, by exposure, at the time of injury, to other war gases besides mustard, and by coincidental but unrelated infections While what we now know to be the principal systemic actions of mustard gas were described during World War I, the conclusions were not presented in a sufficiently clear or consistent pattern to encourage general consideration or acceptance Three fairly complete clinical reports and several minor papers (1, 38,166,232), based on studies of military casualties and accidental mustard burns, were recorded by Allied observers Stewart (207), in a study of 10 severely burned soldiers, observed that those most severely affected developed an early leukocytosis followed by leukopenia within 3 to 4 days Krumbhaar (147–149) independently described the leukopenia in severe mustard gas poisoning and related it to bone marrow injury Herrmann (112) and Warthin, *et al* (218–223), in addition to describing in detail the external lesions produced by mustard, noted erosions and ulcerations in the stomach and intestines of severely gassed individuals These lesions were

thought to be due either to embolic phenomena from infections of the burned areas or to the swallowing of food or saliva contaminated by mustard

Leukopenia from exposure to mustard gas was not observed by the Germans during World War I. Headache, nausea, and vomiting, which occurred a few hours after exposure (49,104,215), gastrointestinal lesions (163,215) and progressive cachexia (49) were described, and there was considerable speculation on the systemic actions of mustard gas (113,225).

The fragmentary clinical experiences of World War I correctly but rather inconclusively implicated the nervous system (headache, nausea, and vomiting), the gastrointestinal tract (nausea and vomiting, anorexia, diarrhea, gastric and intestinal erosions), and the hematopoietic system (leukopenia, anemia, and bone marrow depression), as the tissues particularly sensitive to the systemic action of mustard gas

Laboratory investigations of the local and systemic effects of mustard gas were conducted briefly during World War I. Winternitz and associates (228) exposed dogs to mustard vapor, and studied its necrotizing action on the respiratory tract The toxicity of mustard and related substances was determined by Marshall and Williams (159) Lynch, Smith, and Marshall (155) administered large doses of mustard parenterally to dogs, and described acute effects including salivation, vomiting, and convulsions preceding death Dogs surviving for several hours or longer developed a bloody diarrhea, and showed gross evidence of injury to their intestinal mucosa Long exposure to high concentrations of mustard vapor, or the application of large amounts of liquid mustard to the skin induced similar effects The intestinal injury produced by the parenteral administration of mustard was examined in rabbits by Warthin and Weller (218,220) Pappenheimer (173) and Pappenheimer and Vance (174) observed the development of leukopenia following the intravenous injection of mustard in rabbits, and the associated depletion of the bone marrow and lymphocytic fragmentation

Flury and Wieland (73), in Germany, carried out a detailed study of the acute and delayed toxicologic effects of mustard in several species of animals They suggested that tissue destruction

was one of the systemic effects of mustard gas, because in metabolic studies on mustard-intoxicated dogs they observed a marked increase in the urinary excretion of total nitrogen, ammonia, creatinine, and phosphorus.

Interest in the pharmacology of mustard gas seemed to subside following World War I Several reports on some chemical reactions (54,60,121,150) of mustard gas appeared and, in an interesting review, it was suggested that mustard gas might be of value in the treatment of local cancer, falling hair, and skin diseases (8). Several years prior to World War II there seemed to be a spurt in investigative activity in Europe, as evidenced by the appearance of numerous reports on the toxic effects of mustard gas in animals. Muntsch (167), Maier (157) and Drews (63) applied liquid mustard to the skin of rabbits and guinea pigs and studied the development of leukopenia The former two workers suggested that the blood picture might serve as a good index of systemic intoxication. Telbisz and Kucharik (210,211) produced intoxication by cutaneous application of mustard to rabbits and guinea pigs, and described the anorexia and severe weight loss that preceded death, death, however, was not due to starvation The most detailed study of the pathologic effects produced by the parenteral administration of mustard gas in rabbits was reported by Modderaar (164). The various organs of the poisoned animals were examined, and the stages in the development of leukopenia and bone marrow aplasia, and their recovery in surviving animals were described in detail. It is again worth noting that the systemic effects of mustard gas, which subsequently aroused so much attention, were established and yet largely ignored prior to World War II No appreciation of its systemic effects, for example, can be found in a standard textbook on pharmacology (93) or hematology (62) published prior to 1941

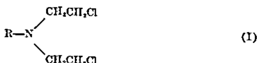
Nitrogen Mustard Ward (217), in 1935, prepared tris(2-chloroethyl)amine, the first compound in the nitrogen mustard series He recognized the similarity between nitrogen and sulfur mustard, and suggested that tris(2-chloroethyl)amine might be useful as a chemical warfare agent Further work did not proceed until the stimulus was provided by World War II Then in rapid succession, England, Canada, and the United States created large-scale projects to study the chemistry, pharmacology and toxicity of chemical warfare

agents, and to devise methods for preventing or treating their injurious effects in man. In the United States, three groups cooperated in this work: *(1) the National Defense Research Committee; (2)* the Committee on Treatment of Gas Casualties of the Committee on Medical Research, both of which were subdivisions of the Office of Scientific Research and Development (these committees arranged for research on special projects through contracts with university and industrial research groups); and *(3)* the Medical Division of the Chemical Corps, U.S. Army, which conducted most of its research in its laboratories at Edgewood Arsenal. This massive effort in the United States was closely integrated with similar work in England and Canada. Over the period of 1940 to 1946, under the strictest military secrecy, an enormous amount of information on the sulfur and nitrogen mustards accumulated Military restrictions were rapidly lifted at the end of the war and, in the past two years, *following the publication of several introductory articles on the* war gases (25,81,84,184), much of the data have been reported in the literature.

From the beginning of the work, it was apparent that the nitrogen and sulfur mustards were qualitatively very similar in their mode of action and pharmacologic effects. Consequently, conclusions derived from experiments with one type of mustard were, when feasible, applied to the related compound, this process obviated duplication In this review of the biologic effects of the nitrogen mustards, data on the sulfur mustards applicable to an understanding of the former, are included with appropriate reservations in interpretation

CHEMISTRY

The nitrogen mustards are bis(2-chloroethyl) substituted tertiary amines of the general formula



A representative compound in this series is a nitrogen mustard with a methyl substituted radical (I), methyl bis(2-chloroethyl)-amine, which is designated by the code name HN2 This is the nitrogen mustard in common clinical use, and it is the one chiefly con-

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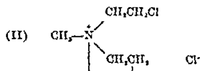
In aqueous solutions, many compounds compete so actively for the imine linkage that its reaction with water is negligible. The reactions of the nitrogen and sulfur mustards have been described by Bergmann and his group (76–78,89–92,165,198–205), Herriott (108,109), Boursnell, Francis, and Wormall (33), du Vigneaud and his group (215b), and Grant and Kinsey (96d,e). Gilman and Philips (84) state, in summary, that the ethylenesulfonium (active form of sulfur mustard) and imine compound (active form of nitrogen mustard) will alkylate the functional groups of many important biologic substances, including: "α-amino, imidazole, sulfhydryl, sulfide, phenolic, ε-amino, and imino groups of amino acids and peptides, the amino group of adenosine and thiamine; the pyridino-N of nicotinic acid amidine and pyridoxine."

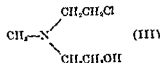
Reactions with Proteins. Mustard gas reacts with a variety of proteins under physiologic conditions (14,50a,102,110,111,143, 175,206a,b,231a). Their free amino groups, with the possible exception of the enzyme, hexokinase, are unaffected (14,111), but the free carboxyl (111) and sulfhydryl groups (14,175) are decreased. Casein, treated with sulfur mustard, will not support the growth of rats, and it was found that histidine, lysine, methionine, and threonine were not available for nutritional purposes (142, 143) The reaction products of sulfur mustard and proteins are practically nontoxic to animals (102,175). The immunologic properties of proteins treated with mustard gas are altered (22,36). Sulfur and nitrogen mustard have also been shown to inactivate complement selectively (37,223a).

Nucleoproteins react with sulfur mustard in alkaline solution to form an insoluble compound containing sulfur which cannot be redissolved, serum protein fractions are not affected similarly (21). Under mild conditions, sulfur mustard reacts with nucleic acids (ribose nucleic acid (yeast) and desoxypentose nucleic acid (calf thymus), the reaction product contains sulfur but not chlorine. Sulfur mustard was found to react with the primary and secondary phosphoryl groups, and the amino groups, when present. The purine-pyrimidine hydroxyl groups have been found to react only in certain cases (70). Pullinger (181) described the formation of an insoluble coagulum in skin treated with sulfur mustard and suggested it was due to the formation of a mustard–nucleoprotein

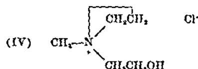
sidered in this report As the free base, HN2 is an oily, volatile liquid (182a), sparingly soluble in water, and actively vesicant and injurious to the eyes and respiratory tract, either as a liquid or as a gas. In combination with hydrochloric acid it forms a stable, white powder which is relatively safe to handle and is very soluble in water.

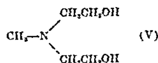
In solution, at *pH* above 7 0, HN2 undergoes rapid intramolecular rearrangement with a loss of a Cl^- to form an imine linkage (II),





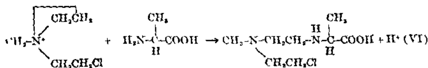
which is highly reactive with many substances. In aqueous solution it reacts with water to form the "chlorohydrin" form (III). The second 2-chloroethyl group then iminizes to form another imine linkage (IV), and this, in turn, reacts with water to form methyldiethanolamine (V) The mustard molecules may also react with





each other to some extent to form condensation products. The reactions of the chloroalkylamines were anticipated by Boyland (40,41a) on the basis of changes occurring in the toxicity of aged alkaline solutions of HN2, and proved by Golumbic *et al* (89,90), by the isolation of the reaction products of HN2 in water as picrosulfonates Kinetic studies on the reactions of HN2 and its derivatives under various conditions have been reported by Thompson and his group (101a,102a,212a–e).

Reactions with Various Chemicals The imine linkage (II) is the chemically reactive form of HN2 In this form it reacts with a variety of chemicals and biological substances As an example, the reaction of the imine form of HN2 and alanine (76) is shown



Mitotic Inhibition and Nuclear Effects Mustard gas inhibits mitosis at doses which do not produce any detectable metabolic abnormality. The inhibition of mitosis may be transient, and some cells recover within 12 to 24 hours; permanent interference with cell division is associated with serious tissue injury. Magne and Remy (156) found that sulfur mustard selectively inhibited the division of yeast cells, and Kinsey and Grant (139–141c) studied the interference with their growth under a variety of conditions. Du Vigneaud and Stevens (215a) have reported the presence of a yeast growth inhibitor, other than bis(2-chloroethyl) sulfide, in mustard gas purified by redistillation Mustard induced abnormal mitoses and polyploidy in barley (72), and interfered with the development and cleavage of marine eggs (153) Friedenwald and collaborators (74,75) carried out an elaborate study of the effects of several nitrogen mustards on the corneal epithelium At threshold concentrations, mitotic activity was transiently inhibited, and it was calculated that 10^6 molecules of mustard per cell were fixed to produce this effect, larger amounts, calculated to be 2×10^7 molecules per cell, produced nuclear pyknosis and fragmentation and cells in the premitotic stage appeared to be more sensitive Koller (145) found marked changes in the pollen seeds of *Tradescantia* exposed to mustard gas, at large doses, breakage and translocations of the chromosomes and delays in mitosis were observed. Mitotic activity in the regenerating livers of partially hepatectomized rats was decreased in rats treated with mustard, although the uptake of radioactive phosphorus was unaffected (158)

Cellular Alterations The concentration of the mustard compounds necessary to injure cells varies widely among the different cell types At doses which are fatal to cells, some undergo pyknosis and nuclear fragmentation, others show nuclear and cytoplasmic enlargement before dissolution, and at sufficiently high doses they all show coagulation necrosis and fixation.

In vitro, nuclear fragmentation and destruction of the nuclear membrane occurs in lymphocytes exposed to nitrogen mustard (138,188) Chick cells were relatively resistant to nitrogen mustard, 0 04 to 0 08 mM solutions of tris(2-chloroethyl)amine damaged proliferating cells, and 0 33 mM killed them. Nonproliferating chick heart muscle continued to contract for 1 day at 0 65 mM

complex. Tris(2-chloroethyl)amine reacts with purines and pyrimidines (85); and the nitrogen mustards depolymerize thymonucleic acid as shown by a decrease in viscosity (52).

Sulfur mustard has been shown to inactivate the rabies, hog cholera, encephalomyelitis (212), Newcastle's chicken virus, tobacco mosaic, rabbit papilloma (Shope), rabbit myxoma, and other viruses (110a), and the nitrogen mustards have been used to inactivate an influenzal virus (187); this inactivation has been produced without impairing the antigenicity as a vaccine or the ability of the influenzal virus to agglutinate chick red cells. The rates of inactivation of the viruses and of bacterial and yeast cells were of the same order of magnitude, and faster than those of enzymes. Viruses containing desoxyribosenucleic acid appeared to be inactivated faster than those containing ribosenucleic acid (110a). An observation of great interest is that bacterial viruses could still form on bacteria (Escherichia coli B and Staphylococcus muscae) presumably dead following treatment with mustard gas at pH 7.5 to 8 (111a)

Reactions with Enzymes in Vitro. Mustard gas will inactivate all enzymes *in vitro* if a sufficient amount is used In attempting to determine the selective sensitivity of enzymes, Dixon and Needham (61) found that hexokinase or the "phosphokinases" categorically were inhibited by low concentrations of mustards. Herriott, Anson, and Northrop (111) observed a gradation in enzyme sensitivity to sulfur mustard, with chicken pepsin, which is easily inactivated, at one end, hexokinase in the middle, and chymotrypsin, which is resistant, at the other end Grant and Kinsey (96c) examined the factors influencing the inactivation of urease, a sulfhydryl enzyme, by sulfur mustard Of the many enzyme systems studied *in vitro* by Guzman Barron *et al* (98), the only ones inhibited at "physiological concentrations" were choline oxidase, choline esterase and choline acetylase The studies of the effects of mustards on enzyme systems *in vitro* have contributed little toward explaining its biologic effects on cells and animals

EFFECTS ON CELLS

The effects of the mustard compounds on single cells and isolated tissues appear to be both highly specific and very complicated.

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and for 5 days at 0.33 mM (57). Fell and Allsopp (72a) studied the effects of sulfur mustard on choroid and sclerotic tissue from the chick embryo growing in tissue culture. At high concentrations the cells were immediately coagulated and killed with little distortion in form. Doses of 50 to 350 gamma per cubic centimeter (0.31–2.2 mM) were the minimal immediate lethal doses for fibroblast cultures *in vitro*, but cells are killed by continuous cultivation in concentrations less than one-seventh of the minimal immediate lethal dose. The cells exposed to the lower concentrations showed enormous distortion of the cell outline, of the chromosomes in mitotic cells, and of the chromatin structure in resting nuclei.

Damaged yeast cells appeared enlarged 8 to 12 hours after exposure, and on recovery they resumed their normal size. The oxygen uptake and carbon dioxide output of those cells were decreased, and no change in cell permeability could be demonstrated (141). It was calculated that 2×10^7 molecules of sulfur mustard per yeast cell were fixed at concentrations which destroyed 40 per cent of the yeast cells (140). Gillette and Bodenstein (80) exposed salamander embryos (*Amblystoma punctatum*) in solutions of HN2 and observed a variety of changes in cellular growth and differentiation. These studies were subsequently described in detail (26, 27). At appropriate concentrations, mitotic activity ceased, the cells being arrested in the interphase state of their mitotic cycle. Some of the cells, including their nuclei and nucleoli, continued to increase in size and to differentiate until they reached an enormous size, following which they broke down. Other cells, which were older, more differentiated and mitotically less active, appeared to be relatively resistant to HN2. In a special study on the developing eye of the salamander, two types of cellular effects were seen following exposure to HN2; some of the cells underwent rapid disintegration, whereas others, located at the eye rim, became enlarged and then degenerated. A similar difference in the response of cells to HN2 was also described in the rat cornea (74,75).

Metabolic Effects. On exposing yeast to mustard containing radioactive sulfur, Kinsey and Grant (141–141c) found that 50 per cent of the mustard reacted with glutathione, 10 per cent with cellular enzymes, some of which were identified as being responsible for carbohydrate metabolism, and 40 per cent with unidentified

structural elements in the cell. The proportion of mustard fixed in these various fractions did not vary with the amount used, implying that there was no substance present in the cell which had an extremely high reactivity with mustard. In bacteria, the respiratory and glycolytic processes were inhibited by sulfur mustard (126) In sufficiently high concentrations, nitrogen mustard inhibited the respiration of a variety of mammalian tissues, but it had little effect on anaerobic glycolysis The respiration of lymphoid tissue was particularly sensitive to HN2 HN2 was shown to inhibit the oxidation of pyruvate and *l*-amino acids, the utilization of ammonia, and synthesis reactions involving the formation of carbohydrates, of creatine, and of urea. Tissues from animals lethally intoxicated with HN2 showed a complete inhibition of choline oxidation, partial inhibition of pyruvate oxidation by the kidney, and urea synthesis by the liver. In rats receiving about 5 times the lethal dose of HN2, choline oxidase was inhibited in the kidney, and partial inhibition of hexokinases and pyrophosphatases occurred (99). In rabbits poisoned with sulfur mustard, there was a rapid decrease in anaerobic glycolysis and bone marrow respiration, which apparently paralleled the decrease in marrow cellularity and modification in cell population (169). Hutchens *et al* (120) studied the effects of tris(2-chloroethyl)amine on the *Chilomonas paramecium* This organism was killed at a concentration of 10 mM. The few clones surviving this exposure showed an attenuated rate of growth and respiration for several months In more dilute solutions, cell division was inhibited; and individual cells, over the period of 5 to 15 hours *after exposure, increased in size and contained a greater* amount and proportion of starch and a smaller proportion of protein as compared with the controls, following which they recovered Oxygen consumption, acetate utilization, and starch synthesis were not significantly depressed, but ammonium utilization and protein synthesis were markedly inhibited. In amphibian embryonic cells exposed to *HN2*, *Bodenstein and Kondritzer (29)* found that the formation of ribose nucleic acid was not inhibited, but its conversion to desoxyribosenucleic acid was blocked While certain specific changes have been observed in cells exposed to mustard, these effects cannot be correlated directly with the nature of the cell injury and the cause of cell death Similarly, the processes in

the cell vital to its function, which are damaged by exposure to mustard, have not been elucidated.

Mutagenic Effects. Of great biologic importance was the demonstration by Auerbach and Robson (11) that sulfur mustard was mutagenic in *Drosophila melanogaster* This was the first example of a chemical which produced mutations, analogous to those caused by x-rays The mutations were produced by treating the *Drosophila* male with mustard gas, and the results suggested that the mustard acted directly on the chromosomes, and not indirectly through the cytoplasm These effects have been analyzed in detail in subsequent reports (9,10,12,96b), and the nitrogen mustards were also shown to be effective mutagenic agents (44a,75b,226a). Mutations have also been induced in *Neurospora* (115,160) and in *Penicillium notatum* (201) by sulfur and nitrogen mustard.

A possible interpretation of the effects of mustard on cells would be as follows: *The mustard molecules gain ready access to the cell* and diffuse throughout it; the sensitivity of the cell to the action of mustard is due to the inherent nature and functional status of the cell rather than to the amount of mustard which penetrates; the specific substances, in the cell, whose reaction with mustard results in its manifold effects
in the conversion of ribose
tion of certain synthetic a
are not known, but it would
among other reactions, alkylates certain substances within the nucleus, perhaps specific nucleoproteins, and this reaction results, in various degrees, in distortions in cell function. The comparison of the effects of x-rays and mustard on cells, which is considered later, contributes to this interpretation

EFFECTS ON ANIMALS

The parenteral administration of the nitrogen mustards to animals and man induces characteristic effects depending on the dosage and route of administration

Acute Pharmacologic Effects The intravenous injection of supralethal doses of HN2 induces a variety of acute pharmacologic effects (5,40,41a,84,176) These consist of *central excitation*, with unsustained convulsions which terminate in death within a few hours; *cholinergic action*, involving both muscarinic and nicotinic

components in a manner similar to the pharmacologic effects of acetylcholine, a *nicotinic action* on the autonomic ganglia, and a *paralytic action* from which the animals develop a progressive weakness of the head muscles, back, extremities, and thorax. The parasympathicomimetic effects of HN2 are evidenced in the injected animal by salivation, defecation, urination, hyperperistalsis, and pupillary constriction; the prophylactic administration of atropine alters some of these effects but it does not have this action if given after HN2 At lower doses, in the range of the minimum lethal one, the animal dies 3 to 7 days after injection; this is referred to as *delayed death* and will be discussed later in detail. The toxicologic effects of other mustard compounds have been compared with HN2 (5,6,84,176).

In view of the chemical transformations that HN2 may undergo under physiologic conditions, it is interesting to examine the toxicity and the pharmacologic effects of these transformation products *in vivo*. The following table (Table I) is adapted from Philips and Gilman (176).

TABLE I

Comparison of Pharmacologic Actions of Methyl Bis(2 chloroethyl)amine and its Transformation Products

	Compound*	Approx LD_{50} mg/Kg	Central excitation	Parasympathicomimetic action	Paralytic action	Delayed death
I	HN2	1–3	+	+	+	+
II	Chloroimine	1–3	–	++	+	+
III.	Chlorohydrin	12–22	–	–	+†	–
IV.	Hydroxyimine	3–5	–	–	+†	–
V.	Diethanolamine	200	–	–	–	–

* See section on Chemistry

† It is likely that these paralytic effects differ from those produced by derivatives I and II This has been investigated by Hunt and Philips (118)

Distribution of HN2 in Vivo There are several experiments which are pertinent to an understanding of the distribution and transformation of HN2 following its injection into animals By occluding the circulation to the hind limbs of rats and rabbits during and for 2 to 15 minutes after the intravenous injection of HN2

proximal to the clamp, Karnofsky, Graef, and Smith (134) showed that the femoral bone marrow was unaffected as compared to the severe injury produced in the sternal and humeral marrow. Similarly, temporary occlusion of the blood supply of a segment of the intestine protected it from the injurious action of HN2. The experiments were not as conclusive when performed with sulfur mustard (134,169). Black and Thomson (23), however, showed that when one dog was injected with a large intraperitoneal dose of sulfur mustard and cross-circulated with another dog 30 minutes later, the first dog died quickly of mustard poisoning whereas the second dog was unaffected Boursnell *et al.* (32,34) injected rabbits intravenously with 5 mg. per kilogram of sulfur mustard labeled with radioactive sulfur (S^{35}). S^{35} left the blood stream rapidly and was distributed throughout the tissues, within 20 minutes it appeared in the bile and urine The liver, kidneys, and lungs had the highest concentration of S^{35}, and this was thought to be due to the excretory function of these organs. The amount of S^{35} fixed in the bone marrow was small, but on a S^{35}/N ratio it was of the same order of magnitude as the corresponding ratio in other tissues. The S^{35} in the tissues could not be extracted easily and it was presumed that it was "fixed" by protein complexes Derivatives of sulfur mustard containing S^{35} were also studied (35)

These data suggest a reasonable interpretation of the distribution of HN2 *in vivo* Following intravenous injection, HN2 traverses the lung capillaries There is no evidence that it reacts with the lung tissue during this passage On passing through the left ventricle of the heart, HN2 is distributed throughout the arterial system, so that within 1 minute some HN2 has reached all the tissues of the body It is possible that much of the HN2 escapes into the tissues and penetrates the cells following this single passage, but it is more reasonable to presume that several circulations are necessary The injection of HN2 into the portal circulation so that it passes through the liver first reduces its toxicity only slightly. In another experiment, HN2 was injected into the abdominal aorta distal to a clamp placed on the aorta and vena cava, and the clamp was left on for 10 minutes Although the mustard thus was pooled in the legs for 10 minutes before being released into the general circulation, it retained most of its systemic toxicity This would

suggest that the competition of certain substances in cells for HN2 as well as its rapid general distribution are factors in the fixation of HN2. It is very probable that HN2 disappears from the blood within 2 minutes after intravenous injection.

When HN2 is introduced into the blood stream, it must be converted to the imine form before it is reactive. The central excitation, however, is apparently due to the HN2 directly. The chloroimine does not induce central excitation, but has a marked parasympathicomimetic effect and produces delayed death. Since the injected HN2 exhibits a mild parasympathicomimetic action, it is likely that only a small amount of it was converted into the chloroimine in the blood stream. Moreover, it has been shown that high blood levels of sodium thiosulfate *in vivo* will protect animals against several lethal doses of the chloroimine by reacting with and detoxifying it in the blood, whereas thiosulfate is only slightly protective against the intravenous injection of HN2. These observations would indicate that HN2 penetrates the cells as such and is then converted into the reactive form (chloroimine). Anslow *et al.* (5) have explained the fact that the injection of the chloroimine form of HN2 produces the same type of delayed death as HN2 by suggesting that the chloroimine is converted to HN2 under the conditions existing in the blood, and it penetrates the cells as such In any event, after entering the cells as HN2 or the chloroimine, the chemical diffuses throughout and then reacts, as the imine form, with various substances The data of Boursnell *et al* indicate that the ratio of S^{35}/N is fairly constant throughout the tissues, with the exception of the liver, kidneys, and lungs It is likely that these latter organs may concentrate degradation products containing the S^{35} rather than reacting with the toxic form of the sulfur mustard.

Delayed Death In the range of the intravenous LD_{50} dose, about 1 to 3 mg. per kilogram in mammals, a single injection of HN2 induces a characteristic pattern of intoxication which may result in death in 4 to 7 days Mice, rats, and rabbits remain relatively asymptomatic for 24 to 48 hours after injection (dogs, however, usually vomit), showing only a mild anorexia and some weight loss, following which they become depressed, stop eating, become weaker and sometimes hyperirritable, develop diarrhea (mild in rabbits, severe in dogs, rats, and mice), rapidly lose weight, and finally col-

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the size of these organs Chanutin and Ludewig (53a) have studied the chemical changes in the rat thymus involuted as a result of treating the animal with HN2. Although adrenal enlargement and associated chemical changes in the gland have been described in rats poisoned with HN2 (134,138,154), indicating that an "alarm reaction" (which in itself produces destruction of lymphocytes) has occurred, it has been shown that the effect of HN2 on the lymphocytes is largely a direct one (134). Degenerative changes appear in the bone marrow within 6 to 12 hours, and myeloid and erythroid elements gradually disintegrate, so that at 48 to 72 hours the bone marrow is almost acellular, consisting chiefly of dilated vessels and a protein coagulum A few reticulum cells persist and, if the animal survives beyond 4 days, the bone marrow recovers rapidly.

Gastrointestinal Effects. The severe diarrhea developing within 24 to 48 hours after the injection of HN2 is apparently due to marked degenerative changes in the intestinal mucosa, which are seen both grossly and on microscopic examination (96). The epithelial cells of the small intestine swell and then slough off, so that large denuded areas appear. In the dog, intestinal hemorrhage is common (116). Gastrointestinal injury has not been found in man even at doses which cause severe bone marrow injury (195).

Effects on Other Tissues The gonads have not been studied in detail, but there is no doubt that they can be injured by HN2 and testicular injury probably occurs in human males receiving therapeutic doses of HN2 (195). Histologic examination of other organs has revealed no consistent or significant injury which might contribute to the toxicologic effects of HN2 (96,195).

Effects in Pregnancy. Since the nitrogen mustards have a marked influence on embryonic development, and resemble x-rays in their biologic effects, there is good reason to expect them to produce changes in the mammalian fetus Haskins (102a), in a much-needed study, has observed deformities in the fetuses of rats treated during pregnancy

Metabolic Effects There have been several observations on the metabolic and biochemical changes in animals poisoned with mustard gas Lethal doses of nitrogen mustard, administered by several different routes to dogs, did not alter the electrophoretic pattern of the serum proteins, but, just before death, a fall in the

lapse just before death. Most of the animals surviving for 7 days after injection went on to complete recovery. Occasionally, an animal may be so severely debilitated by the treatment that it succumbs to secondary infections (50,96,138).

Hematologic Effects. Animals intoxicated with HN2 exhibit a marked alteration in their peripheral blood picture. Within 24 hours after injection, there is a marked decrease in the number of lymphocytes, and Cameron *et al.* (50) reported that there is also a decrease in the lymphocyte count in lymph obtained from the thoracic duct A leukocytosis may appear within 24 hours due to a transient rise in granulocytes. During the next 2 to 3 days, however, the granulocyte count falls, so that 3 to 4 days after injection, when the intoxicated animals begin to die, the total white count is less than 500 cells per cubic millimeter. Those animals surviving beyond 4 days show a rapid recovery of the white count, and the lymphocytes may recover more quickly than the granulocytes. At sublethal doses, the white cell count is depressed 2 to 3 days after injection, and then it recovers quickly (96).

The red cell count is not greatly altered in intoxicated animals, but the reticulocytes are severely depressed (50). The formation of red cells is interrupted but, because of the long life of the red cell, this is not immediately reflected in the red cell count. The platelets in animals given HN2 have not been studied extensively, but Jacobson *et al.* (121a) have observed a moderate fall in the platelet count of rabbits receiving the maximum tolerated dose of HN2. In man, a toxic dose of HN2 will produce a marked leukopenia, thrombocytopenia, purpura, and severe anemia within 7 to 10 days This toxic syndrome will be discussed later Smith *et al* (192) have found that the coagulation time is prolonged, due to the appearance of an anticoagulant in the blood This has also been observed in rabbits intoxicated with HN2 and with x-rays (121a) This substance is indistinguishable from heparin and is counteracted by protamine or toluidine blue

The changes in the cellular elements of the peripheral blood are closely paralleled by injury to the hematopoietic organs The first changes, which occur 5 to 10 hours after injection, are pyknosis, fragmentation, and disintegration of the lymphocytes in the thymus, spleen, and lymph nodes, followed by a marked shrinkage in

8 to 9 days after exposure. The former group appeared to die in a shocklike state, a severe leukopenia developing in those surviving 3 to 4 days The second peak of deaths was apparently caused by infections in severely debilitated individuals

Effect of Antibody Formation. Hektoen and Corper (105) described a reduction in antibody formation in animals treated with sulfur mustard Philips, Hopkins, and Freeman (177) immunized goats with a potent antigen, ricin, treated the goats chronically with tris(2-chloroethyl)amine in sufficient dosage to produce a severe leukopenia and then measured the response to a stimulating dose of the antigen The anamnestic response was delayed in the mustard-treated animals, although the same titer as that reached by the controls was finally achieved. The Schwartzman reaction in rabbits was completely suppressed by pretreatment with HN2 and with benzol (15a).

Cumulative Action. The administration of HN2 by rapid injection or by extending the period of injection over 6 or 8 hours does not appear to alter its toxicity (132). The drug is fairly cumulative in its toxicity. Over a period of 1 month, a total of 5.5 mg per kilogram of HN2 in rabbits (about twice a single fatal dose) produced weight loss, a severe depression of the leukocytes and reticulocytes, and a mild anemia The clinical condition of the animals improved and the peripheral blood counts promptly returned to normal after the cessation of HN2 (50). In rabbits, it was found that the daily administration of 1/7 of an acute LD_{50} dose of HN2 was cumulative; 1/10 of the LD_{50} dose produced a progressive and severe weight loss, diarrhea, and a moderate leukopenia within 6 to 8 weeks, which sometimes proved fatal, and 1/14 of an LD_{50} dose had little effect over a period of 2 months (132) The normal bone marrow of man recovers in 4 to 6 weeks from a toxic dose of HN2, if recovery from one course is complete it shows a normal response to a subsequent course In patients given repeated courses of HN2 there is no definite evidence, if suitable intervals between courses of treatment are allowed, for a cumulative injury to the bone marrow (196)

Effects on Other Species Although the mammals studied showed no great differences in their susceptibility to HN2, chickens have proved to be relatively resistant to the drug (96) The acute LD_{50}

albumin, a rise in the alpha-globulin fractions, and a change in the appearance of the beta-globulin fraction occurred (86). A rise in plasma fibrin, cholesterol, and blood sugar was also found; the elevated cholesterol was attributed to the formation of an abnormal lipoprotein (53). Exposing the body of the dog to lethal doses of mustard vapor induced a hyperglycemia, followed by a profound hypoglycemia just before death (65). These changes could be induced by a variety of nonspecific methods destructive to tissue (51) The serum of rabbits injected with HN2 becomes more favorable to the growth of bacteria, beginning about 6 hours after injection (134). The significance of this observation is not known.

Dogs given lethal doses of tris(2-chloroethyl)amine (applied to the skin) showed a great increase in water intake and urine excretion; excessive loss of sodium, potassium, and chloride in the urine; and a decrease in intracellular and extracellular fluid. There was also an elevation in the urinary excretion of nitrogen and phosphorus, indicating an increase in tissue destruction (178). Houck *et al.* (116) observed in dogs poisoned by the intravenous injection of nitrogen mustard a reduction in intracellular and extracellular fluids, a decrease in plasma chlorides, an increase in carbon dioxide capacity and blood *pH*, and a fall in circulating plasma protein and red cell mass. Before death, 3 to 5 days after injection, the dogs were weak, comatose, hypotensive, hypothermic, and there was a decrease in the oxygen saturation of the arterial blood. It was suggested that death was a consequence of circulatory failure precipitated by a reduction in blood volume attributable to a loss of protein, electrolytes, and water through vomitus and diarrhea Attempts at therapy by fluid and electrolyte replacement were unsuccessful, however Protection of the small intestine by a temporary occlusion of the circulation during and for 15 minutes following the injection of the nitrogen mustard prevented the severe diarrhea and decreased the vomiting, but the survival time of the dogs was only slightly prolonged

A large number of men exposed to an oil – sulfur mustard mixture during a sea disaster, were studied by Alexander (3) Mustard was apparently readily absorbed through the skin and, of 617 casualties with some degree of mustard injury, 83 died Most of the deaths occurred during two peaks, one to 4 days and the other

tion and fixation of sulfur mustard in the skin (13,190), and a chemical study on the rate of cutaneous penetration of sulfur and nitrogen mustard (168) are pertinent to a consideration of this problem. It should be noted, however, that whereas the parenteral administration of nitrogen mustard does not produce sensitization to further injections, cutaneous sensitization is a common consequence of topical application of mustard. Fell and Allsopp (72b) compared the effects of repeated applications of sulfur mustard or benzopyrene (a carcinogen) to mouse skin. Although both agents produced skin damage, cellular abnormalities, and hyperplasia, the mustard-treated skin showed no evidence of neoplastic alteration.

EFFECTS ON TUMORS

In 1931, Adair and Bagg (2) demonstrated that the local application of sulfur mustard to superficial tumors in mice would induce regressions. Some satisfactory clinical results were also obtained in 13 patients with superficial cancers treated by local application or intratumoral injection of sulfur mustard, but the technical difficulties in this method of therapy discouraged further work. Sulfur mustard was subsequently found to induce tumor regressions in several types of mouse and rat tumors (128,216), and tumors of lymphoid origin were particularly sensitive to its action (15).

The nitrogen mustards were also found to interfere with the growth of tumors in laboratory animals Boyland *et al.* (41–43), using HN2, produced regressions of a spontaneous mammary carcinoma and a transplantable lymphosarcoma in mice and of the Walker carcinoma in rats The tumors usually recurred but the lives of the animals were definitely prolonged A single dose proved more effective than the same total dose spread over a period of days Nuclear abnormalities and mitotic inhibition were described in the Walker carcinoma growing in rats treated with HN2 Burchenal *et al* (46–48), by the use of HN2, was able to prolong definitely the lives of mice carrying an active transplantable leukemia

The nitrogen mustards have induced regression in a transplantable mouse thymoma (217a) Bass and Feigelson (14a) have demonstrated that HN2 will produce regression of a lymphoid tumor in mice in the absence of the adrenals This indicates that HN2 damages the tumor directly, rather than through the mediation of an "alarm reaction."

dose produced an early death associated with neurologic manifestations; if the chicken survived this acute effect it usually recovered A mild leukopenia developed, but there was no evidence of gastrointestinal injury. The reason for the resistance of the chicken is not known.

Cause of Death. The tissues sensitive to the *in vitro* injection of HN2 at the minimum lethal dose are the lymphatic tissues (thymus, spleen, and lymph nodes), the bone marrow, and the intestinal mucosa. On the assumption that HN2 is distributed relatively evenly in the body, the above tissues are the ones that are most severely affected at a given concentration of HN2. One thing that these tissues have in common is a high rate of cell division. This is apparent for the hematopoietic organs, but mitoses may be equally active in the intestine. It has been estimated, for example, that the epithelium in the ileum of the rat renews itself every 1.35 days (206). There is no proof, however, that mitotic activity and sensitivity to HN2 are directly related; it may be that tissues which have the function of rapid regeneration or the capacity for growth are also sensitive to the nitrogen mustard. That mitotic activity, *per se*, is not the reason for the sensitivity of the erythroid elements of the bone marrow to x-rays has been shown by Jacobson *et al.* (122).

The cause of death in animals poisoned with mustard remains largely an unsolved problem It is not due to the leukopenia, as such (134), to secondary infection, to tissue-destructive substances liberated by the action of HN2 (134), or to lethal toxins in the blood (23) In man, the hematopoietic injury with thrombocytopenia and bleeding may be adequate to account for death Animals, in contrast, die more quickly than man after minimum lethal doses of HN2, and they also develop intestinal lesions which do not occur in man This would suggest that the intestinal injury has contributed heavily to the intoxication in animals Protection of the intestinal tract from nitrogen mustard injury, however, failed to alter the lethal effects of the drug (116).

Effects on Skin The local actions of mustard gas are not considered in this review There has been interest, however, in the application of mustard to the skin, as a liquid or vapor, for the treatment of certain skin diseases Two histologic studies on the localiza-

mustard therapy have been found in reticulum cell sarcoma, lymphosarcoma, Hodgkin's disease (24a,195), and mycosis fungoides (107).

Metabolically, the exposure of tumor tissue to sulfur mustard *in vitro* is reported to cause a reduction in glucolysis (20) and a reversion toward normal in its metabolism (189). Sarcoma 180 tissue slices, exposed to HN2 *in vitro*, showed a progressive decrease in respiration and aerobic lactic acid formation (125). Even if the tissue is exposed for only 15 minutes, on transfer to an HN2-free environment the metabolic changes progress Boyland *et al.* (42) found a decrease in glucolysis in the Walker carcinoma obtained from rats treated with HN2.

Berenblum (16–19) was unable to detect any carcinogenic activity in sulfur mustard, but when it was applied in association with a carcinogen it interfered with the production of tumors. Boyland (39) has suggested, however, that the chronic parenteral administration of nitrogen mustard may prove carcinogenic in mice

Johnson (127) found that nitrogen mustard exerted a favorable influence on the course of leukosis in chickens

NITROGEN MUSTARD DERIVATIVES

The nitrogen mustard molecule lends itself to a variety of structural modifications (75a, 100a) The distinctive pharmacologic and pathologic effects of the nitrogen mustards make a study of these derivatives of particular interest.

A variety of acute pharmacologic effects can be produced by various changes in the nitrogen mustard molecule The "chlorohydrin" and "hydroxy imine" derivatives of HN2 (*see* formulas III and IV in the section on chemistry) have a paralytic action in animals which is reversible (5,176) Certain mono-2-chloroethyl amines, including *N,N*-dibenzyl(2-chloroethyl)amine, are sympatholytic agents (103,117) 2-Chloroethylmorpholine and 2-chloroethyldimethylamine have been shown to induce severe and prolonged neurologic injury (87,146) Alterations in the unchlorinated chain of the bis(2-chloroethyl)amine group may produce marked differences in the neurologic effects but only quantitatively alters the delayed toxicologic effects (5,88,176)

The tissue injury produced by the nitrogen mustards is the important action from the point of view of cancer chemotherapy This

In tissue culture, the observable responses of normal and neoplastic tissue to tris(2-chloroethyl)amine are qualitatively similar, but different tissues are affected at different dose levels (57). The mouse sarcoma 180, growing on the chorioallantoic membrane of the 12 day old chick embryo, is destroyed by a dose of HN2 which is not toxic to the chick embryo (132). The administration of a dose of HN2 larger than 4 to 6 times the LD_{50} dose to mice carrying a leukemic tumor will usually, within a few minutes, destroy its viability. This "cytocidal dose" which represents the dose level of mustard necessary to inactivate the tumor or, theoretically, to "cure" the disease, will undoubtedly vary with different types of tumors. The "cytocidal dose" for the mouse sarcoma 180, for example, is about 10 to 14 times the LD_{50} dose (132). Groups of mice, each carrying a different type of transplantable tumor ranging from leukemia and lymphosarcoma to a melanoma, were injected with 5 times the LD_{50} of HN2, and sacrificed 3 days later, when they were near death The tumors showed various degrees of regression and cytologic changes The leukemia and lymphosarcoma almost completely regressed and, on histologic examination, the cells were pyknotic and fragmented; the sarcoma 180 regressed partially, and the cells were enlarged and showed nuclear abnormalities, the fibrogenic and osteogenic sarcoma and melanoma regressed very slightly and only modest cellular changes were found (136) It is concluded that neoplastic cells, as normal cells, show a spectrum of susceptibility to HN2. The place that a tumor occupies in this spectrum is due to a level of activity which may be represented in some types of normal tissues, and not to its neoplastic properties, as such

In man, temporary reductions in the size of tumors have been produced with HN2 Histologic changes have been difficult to detect because relatively small doses of HN2 are used and, in spreading the course of treatment over several days, damaged cells may recover or degenerate and disappear before the follow-up biopsy is taken. Thus, one report denies the occurrence in tumors of histopathologic changes which can be attributed to nitrogen mustard (56), although others have described specific alterations and degenerative changes in tumor cells following HN2 therapy (4,119, 195). The most extensive histologic changes associated with nitrogen

analyzed on a subcellular and physical basis, but only its superficial and practical aspects can be considered here.

The Mechanism of X-Ray Action. The fundamental action of x-rays on cells has recently been the subject of several critical reviews (79,151,182,194). Giese (79) has summarized the position of two opposing schools of thought.

(*1*) *Indirect action.* The effect of x-rays on cells is indirect, acting through the formation of active radicals in water. Weiss (226) has suggested that these radicals include OH^- and H^+ ions, H_2O_2 molecules and H and HO_2 radicals, and other active groups. These groups are formed throughout the cell, and systems sensitive to oxidation or reduction may be inactivated by them.

(*2*) *Direct action or target theory.* The effects of x-rays are due to direct hits on chromosomes and genes, and damage to these structures, particularly in cells which are mitotically active, is ultimately responsible for cell death. This view has been supported most vigorously by Lea (151).

The theory of the indirect action of x-rays appears to be the more attractive one for explaining the effects of x-rays on cells On this basis, x-rays induce ionization and the formation of active radicals of water throughout the cell The groups react with each other or with substances in their immediate environment. Approximately the same degree of ionization occurs in all cells, but some cells are much more sensitive than others to a given degree of ionization This sensitivity may be due to absence of protective substances in the cell, or to the presence of special systems whose inactivation is fatal to the cell The nature of the cellular injury produced by x-rays is not known

Similarly, it may be postulated that HN2 diffuses throughout the cells, and because of the absence of protective substances or the presence of special systems vital to the cell and easily alkylated, the sensitive cells are destroyed This would imply that the systems affected by HN2 and x-rays in the cells may be similar, but there are no data on this

Effects on Tissues and Animals The parenteral injection of HN2 and total body irradiation produce the same pattern of tissue injury (hematopoietic organs, intestinal tract, and gonads) and types of delayed death (42,96,144). There is an important differ-

action appears to require the presence of at least two bis(2-chloroethyl) groups, or a closely associated structure, i.e., bis(2-bromoethyl) or bis(2-chloropropyl) groups (5,176). The molecule may be made larger by lengthening the unchlorinated side arm or combining it with other organic chemicals including another nitrogen mustard as, for example, SK 136 (1,3-propanediamine-*N*,*N*,*N'*,*N'*-tetrakis-(2-chloroethyl)dihydrochloride) (175). The ability to form an imine linkage is essential to the "cytotoxic" action, but not all nitrogen mustards which iminize are "cytotoxic."

An important problem is to determine if the pattern of "cytotoxic" injury produced by the nitrogen mustards can be altered or the effects directed toward particular tissues. It is possible, by altering the unchlorinated side chain of HN2 to produce, at LD_{50} doses, minor changes in the pattern of damage among the susceptible tissues. One compound, for example, may cause more bone marrow injury in relation to gastrointestinal injury than another (96, 138). The pattern of cell damage produced by exposing developing salamander eyes to several different nitrogen mustards was essentially the same (28). Burchenal *et al* (47) found, in testing a number of nitrogen mustards for therapeutic activity against mouse leukemia, that those substances which have a structure compatible with "cytotoxic activity" are also therapeutically effective. Of the many nitrogen mustard derivatives tested, none proved more effective than HN2 (189a).

It does not appear likely that, by structural alterations in the molecule, the cytotoxic activity of the nitrogen mustards can be directed toward certain tumors or toward neoplastic cells. There is a definite possibility, however, that a derivative of HN2 may be found for clinical use which does not possess its unpleasant acute pharmacological effects

COMPARISON OF BIOLOGICAL EFFECTS OF X RAYS AND NITROGEN MUSTARDS

There is a striking similarity between the biologic effects of x-rays and the nitrogen mustards (84,96), and the latter have been referred to as radiomimetic agents (41) A comparison of the mechanism of action and the biologic effects of these diverse agents is a project of fundamental interest. This problem ultimately will be

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These studies indicate that a variety of tissues in mice shows the same spectrum of sensitivity and the same type of response to HN2 and to x-rays.

From clinical observation, there is a suggestion that x-rays, but not HN2, may sometimes induce edema in tumors and that x-rays may thus differ from HN2 in being injurious to the vascular system

Dosages The single intravenous LD_{50} dose of HN2 in animals ranges between 1 and 2 mg. per kilogram, and it is estimated as about 1 mg. per kilogram for man. By total body exposure, the LD_{50} for x-rays is about 400 to 600 r. In man, therefore, by a direct calculation, 0 1 mg per kilogram of HN2 is equivalent to total body irradiation with 40 to 60 r. This calculation (absolute effect) is based on the premise that the toxicologic effects of HN2 and x-rays have the same temporal relationship However, in order to produce a temporal distribution of deaths similar to HN2 (immediate effect), about twice the LD_{50} of x-rays (800 to 1200 r.) are necessary. It may thus be postulated that the "immediate effect" of 0 1 mg. per kilogram of HN2 is equivalent to 80 to 120 r. (twice the "absolute effect") However, in relation to the "immediate effects," the action of x-rays is more prolonged and profound, whereas the recovery from HN2 is fairly rapid. At equivalent "absolute effect" doses, x-rays would produce much less striking effects than HN2. An analysis of the comparative time of onset, depth of injury, and rate of recovery of tissues from various doses of HN2 and x-rays is very important, but the scanty data available do not warrant further discussion

Combination of X-Rays and HN2. In mice, using death as the criterion of effect, it was found that 60 per cent of an LD_{50} dose of HN2 followed within 1 to 2 hours by 60 per cent of an LD_{50} dose of total body irradiation was fatal to most of the mice within 4 to 7 days This represented an additive toxic effect, and the mice died during the nitrogen mustard period of death. Giving the same dosage in the reverse sequence, however, resulted in less than an additive effect, and the mice died 6 to 11 days after exposure—a period when most of the deaths occur after LD_{50} doses of x-rays (132). These data indicated that greater injury to tumors might be produced by giving parenteral HN2 prior to local irradiation, rather than in the reverse sequence. The observation that x-rays following

ence, however. Mice receiving a minimum lethal dose of HN2 died within a period of 4 to 7 days; at LD_{50} doses, those animals surviving beyond 7 days rapidly recovered A minimum lethal dose of x-rays to mice was usually fatal within 7 to 15 days after exposure At LD_{50} doses, some mice may die as long as 30 days later and those surviving recover slowly (69,132). At doses of HN2 and total body irradition producing a severe depression in the white cells in the peripheral blood, the recovery of the white cell count following HN2 occurred more promptly (64,132).

It is of interest that Auerbach *et al.* (12) have found some differences between the mutations in *Drosophila* induced by sulfur mustard and x-rays. Mosaics were much more frequent among the progeny of mustard-gas-treated males, and the gonads of mustard mosaics frequently contained both normal and mutated cells, which is rare among x-ray mosaics. A suggested explanation is that a mustard-treated gene does not mutate at once, but has a latent tendency to mutate which may be expressed later

These observations indicate that there is some difference in the effects of x-rays and mustard on cells The more rapid onset of intoxication, and the earlier recovery of animals poisoned with HN2 as compared with x-rays suggest that HN2 only damages a portion of the cells in susceptible tissues (an all-or-none effect), or that cells partially damaged by HN2 recover more rapidly.

Mouse sarcoma 180 growing on the chorioallantoic membrane of the chick embryo is destroyed by a dose of HN2 which does not injure the embryo (132) On the other hand, doses of x-rays to the tumor-bearing egg which destroy the sarcoma 180 are also lethal to the chick embryo (135) Furthermore, the LD_{50} of x-rays for eggs, adult chickens, and mammals is approximately the same Thus, chick tissue appears to be much more resistant to HN2 than mouse tissue, whereas their susceptibility to x-ray seems to be approximately the same These differences are worthy of further study

Effects on Tumors The effects of equivalent supralethal doses of HN2 and x-rays were studied on several different types of transplantable mouse tumors and on the normal tissues of the carrier mouse The types of tumors injured and the degree of regression in the tumors, and the histologic changes in the normal and tumor tissues produced by HN2 and x-rays were essentially the same (136)

GENERAL CONSIDERATIONS

Certain general observations derived from the laboratory and clinical studies on the nitrogen mustards facilitate an interpretation of the clinical data. Some of these statements may appear dogmatic, but they will lend coherence to the subsequent discussion concerning the indications for HN2 therapy.

(*1*) The nitrogen mustards do not have a specific or selective toxic effect on tumor cells, *per se*. The normal tissues vary in their susceptibility to HN2, ranging from the sensitive lymphatic and hematopoietic tissues at one end to the resistant muscle, kidney, and liver tissues at the other. A similar range of sensitivity also exists among the different types of tumors The sensitivity of a given tumor is not related to its neoplastic properties, but to such factors as the tissues from which the tumor originates, the stage of differentiation of the cells, and the degree of mitotic activity.

(*2*) It is possible that some tumors might be more sensitive than normal tissues to nitrogen mustard and, under these circumstances, HN2 could be used to obliterate the tumor. There is no record, however, of a cancer patient who has been cured by the use of HN2. In some cases of lymphosarcoma and allied disorders, the tumor may be somewhat more sensitive than the hematopoietic system, and HN2 may induce almost complete regressions for long periods of time, whereas the bone marrow is only slightly affected and recovers rapidly. Most tumors, however, are more resistant than the bone marrow, and the maximal feasible dose of HN2 will have no useful therapeutic action. The rapidity of tumor regression following HN2 therapy bears little relation to the duration of the regression. Tumors in which rapid and striking regressions have been produced have recurred within 10 days. The rate of regression following adequate HN2 therapy is an index of tumor sensitivity, the duration of the regression is an index of the rapidity of the tumor growth.

(*3*) Tumors, even those responding early in the disease, eventually become resistant to HN2. This may be due to the destruction of the HN2-sensitive cells, whereas the resistant ones survive and grow.* It is more likely that the natural course of each cancer is

action of HN2 could be demonstrated (135).

a sublethal dose of HN2 enhanced the HN2 effect and that, when given in the reverse sequence, the x-ray effect was enhanced (but to a lesser degree), is of great interest. Much work is necessary to determine the dosage, time interval, and sequence of administration of x-rays and HN2 to produce maximal injury to tissue.

Clinical Applications

HISTORY

Gilman and co-workers (83), late in 1942, determined the intravenous toxicity of tris(2-chloroethyl)amine hydrochloride in 6 patients with inoperable cancers. They described a toxic hematologic syndrome consisting of leukopenia, thrombocytopenia, bleeding, and anemia, but also noted tumor regressions and temporary clinical improvement in some of the patients. Shortly afterward, the use of methyl-bis(2-chloroethyl)amine hydrochloride (HN2) in the treatment of Hodgkin's disease was initiated by Jacobson *et al.* (123) at the University of Chicago, and clinical studies were subsequently undertaken at the University of Utah (94,95) and Memorial Hospital, New York (133). This work was shrouded in secrecy until the end of World War II. Although the security restrictions were then lifted, the toxicity and poorly understood indications for the use of the nitrogen mustards made it inadvisable to release them for general use The Committee on Growth, with the cooperation of the Chemical Corps of the U.S. Army, and Merck and Company, organized a program which permitted the wide but controlled distribution of HN2 for clinical trial.

The data compiled in the wartime clinical studies of the nitrogen mustards were summarized in 1946 in an official statement by the Committee on Growth (183), and the detailed reports were subsequently published (94,95,123,133) Under the aegis of the Committee on Growth, Merck and Company packaged and distributed HN2 provided by the Chemical Corps to qualified clinical investigators (185) Data on treated cases were periodically sent to the Committee on Growth, which, in turn, prepared several interim analyses of the submitted cases for use by the cooperating institutions (194) In the past 2 years, the nitrogen mustards have been discussed editorially (66–68,129,152a,229) and have been considered in detail in reviews on the chemotherapy of neoplastic diseases (22a,44,58,78a,82,100,102,124,130,186,197)

tient, it is thus possible to give larger therapeutic doses of HN2 than total body irradiation

(c) HN2 is inexpensive, and easier to handle and administer than x-rays A course of therapy may be given quickly and repeated at appropriate intervals

(7) There are suggestions that HN2 is effective in patients no longer responding to x-ray therapy, and that "radioresistant" tumors once again become radiosensitive following HN2 (94,95, 123) A literal interpretation of this observation would imply that there is a dissociation in the susceptibility of some cells to x-rays and HN2, a contradiction of the principle that the susceptibility of cells to these agents is parallel. More reasonable explanations are possible, however. The term "x-ray fast" is not an absolute one; some patients called "x-ray fast" by one radiologist may be successfully treated with x-rays by another, these patients may also be responsive to HN2 therapy. Secondly, in widespread lymphomatous disease, systemic intoxication may be due to a hidden focus of activity, x-rays applied elsewhere do not produce a remission, but a systemic form of therapy will be successful by ferreting these areas out If the patient shows general improvement, the superficially located disease may then respond to adequate x-ray therapy.

DOSAGE AND TOXICITY

The toxic and therapeutic doses of HN2 are almost the same. A thorough understanding of its toxic effects is therefore necessary to work out an adequate but safe dosage for a given patient.

Method of Administration. HN2 is a white, crystalline, hygroscopic, water-soluble powder It is prepared in a 20 cc. rubber-capped glass vial containing 10 mg. of dried HN2 Just prior to use, 10 cc of distilled water or physiologic saline are injected into the vial, and a solution containing 1 mg per cubic centimeter of HN2 is thus prepared. The appropriate dose is then withdrawn from the vial Although this solution may be stable for several weeks, it is advisable to use a fresh vial for each injection.

The solution of HN2 must be given intravenously. It is usually given rapidly, since the rate of injection appears to be of no importance. There are no symptoms during or immediately following the injection, although rarely a patient may note a peculiar taste. Extravasation of HN2 is usually painful immediately, but the absence of pain is not a certain indication that the injection is going

predetermined in the first neoplastic cells Patients in the early stages of the disease have the longer remissions, those with rapidly advancing disease the shorter remissions, and those in the terminal stage of the disease are resistant to HN2 therapy. The ability of HN2 to interrupt or palliate the course of a cancer will consequently depend to a great extent on its natural course and the distance it has progressed before HN2 therapy was instituted.

(4) The margin of safety in HN2 therapy is small, but the most satisfactory and prolonged clinical remissions are produced by giving the maximum doses of HN2 that can be administered without toxic complications. The rapidity with which a course of HN2 is given and the degree of bone marrow damage hazarded is dependent on the clinical situation.

(5) The extent of tumor regression and the duration of the remission, in relation to the bone marrow injury produced by HN2 therapy, may often be of prognostic value. The correlation of the type and histologic structure of a tumor and its response to HN2 is of interest, and may provide the basis for a classification or new interpretation of certain types of tumors.

(6) The closest counterpart of intravenous HN2 therapy is total body irradiation Medinger and Craver (161) reviewed the results of total body irradiation (Heublein therapy) in a total of 270 patients with various types of neoplastic disease The dose given ranged from 75 to 300 r. over a period of several days The treatment resulted in subjective and objective improvement in the majority of patients with Hodgkin's disease, lymphosarcoma, leukemia, and polycythemia, patients with metastatic carcinoma showed little response Although the clinical effects of HN2 and total body irradiation appear to have much in common, HN2 therapy offers several advantages

(a) The effects of HN2 appear more quickly than those following x ray therapy, but the remissions may be shorter Rapid relief is desirable, however, and it is then possible to apply x rays locally to the areas of resistant disease

(b) The maximum tolerated dose of HN2 can be approximated and administered more safely than a similar amount of x rays. The leukopenia from x ray therapy develops slowly and recovery is delayed Consequently, whereas the white cell count can be brought down with relative safety to 1,000 with HN2, this is rarely hazarded with total body irradiation With less hazard to the pa

tient, it is thus possible to give larger therapeutic doses of HN2 than total body irradiation

(c) HN2 is inexpensive, and easier to handle and administer than x-rays. A course of therapy may be given quickly and repeated at appropriate intervals

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satisfactorily. If any HN2 extravasates, within 24 hours the local area becomes raised, swollen, red, tender, and painful, and this condition may persist for several days. The veins in this area frequently become thrombosed. The edema subsides in 3 to 5 days, and the area then becomes indurated and pigmented, and finally it may go on to fibrosis and contraction. Ulceration from the extravasation of HN2, and embolic phenomena from the venous thrombosis of the arm veins have not occurred in our experience. Some clinics give the HN2 directly into the tubing of an intravenous infusion. While more cumbersome, this technic decreases the incidence of HN2 extravasation and venous thrombosis (229a). In patients receiving many courses of HN2, thromboses of the accessible veins are common complications

Nausea and Vomiting. Nausea is produced by 80 to 85 per cent of the injections of HN2, and often episodes of vomiting occur, beginning within 1 to 8 hours and lasting for several hours (42, 133). This reaction seems to be less severe with later injections in a course of treatment but, in some cases, the nausea and vomiting may persist during the course and for several days afterward. Except for patients with bleeding tendencies, the nausea and vomiting have not been attended by serious consequences. A cerebral hemorrhage and diffuse extravasation of blood into the upper trunk and face, apparently due to severe retching, followed injections of HN2 in 2 adult patients with acute leukemia and bleeding tendencies. Sedatives given shortly after an injection of HN2 may alleviate the nausea and vomiting, pyridoxine is reported by some patients to be helpful, atropine is without value Wintrobe *et al.* (229a,230) have suggested that HN2 be given in the evening in conjunction with a sedative, and the tendency to nausea and vomiting may not be evidenced during sleep The gastric symptoms are the chief deterrents to the use of HN2 in the Outpatient Department By making previous arrangements so that the patient can reach home within an hour after an injection, many patients have been treated without incident on an outpatient basis

Some patients complain of weakness during a course of treatment, and 5 per cent of the patients of Boyland *et al.* complained of lightheadedness and drowsiness following an injection (42) Diarrhea has rarely occurred and it is difficult to relate it to the HN2. Zanes, Doan, and Hoster (231b) have noted a maculopapular

pruritic skin eruption over the trunk and extremities in 13 patients following HN2, but this reaction has not been conspicuous in other clinics Other complications have also been described but, in view of their inconstant occurrence and the fact that these patients have many complaints and are often desperately ill, HN2 cannot be definitely implicated

Hematologic Effects. In most patients, a course of 0.4 mg. HN2 per kilogram of body weight produces a moderate leukopenia, the leukocyte count falling in the range of 2,000 to 5,000 in 7 to 10 days and then recovering within 1 to 2 weeks. The lymphocytes usually decrease in number before the granulocytes, but the differential white cell count is not greatly altered. The hemoglobin level may decline slightly, and the platelet count is generally unaffected (7, 94,95,123,133,196). The hematologic picture is usually restored to normal within 4 weeks In diseases involving the bone marrow and in patients in very poor condition the hematologic effects of HN2 are more variable.

With a sufficiently large dose, and this may range from 0 4 to 1 0 mg per kilogram over a 1 week period for different patients, a severe depression of the bone marrow is produced, resulting in a characteristic toxic syndrome (83,94,95,123,133) Within 7 to 10 days, the leukocyte count falls to below 1,000, the platelets may drop to less than 50,000 and, in mild intoxication, a shower of petechiae may appear over the legs and trunks In these patients recovery is rapid In the more serious cases, a generalized purpura with bleeding from the gums, mouth, and rectum may develop, and associated with this is a rapid drop in the hemoglobin level, and fever running up to 103 F Smith *et al.* (192) have found a prolongation of clotting time in this condition, presumably due to an increase in heparin in the blood. This syndrome appears 7 to 14 days after HN2, and the patient may either die promptly or show evidence of recovery within a week. A rise in the white count is a good prognostic sign The only useful treatments are blood transfusions to correct the anemia, antibiotics to ward off infection and, possibly, protamine to decrease the clotting time. A picture of agranulocytosis has been described in only 1 patient (211). Occasionally, death due to bronchopneumonia may occur, while the bone marrow is showing evidence of active regeneration

Some patients have received more than 15 courses of HN2 over

a period of 2 to 3 years, and this entails a total dosage of 300 to 400 mg. There has been no conclusive evidence of a cumulative injury to the bone marrow (196), if the white count has been permitted to return to normal before further treatment. Some of the patients showed bone marrow depletion, but this was often due to the disease itself, and could not be definitely attributed to HN2. In the presence of a leukopenia or evidence of bone marrow replacement by abnormal cells, HN2 should be given cautiously.

Dosage. The usual course of HN2, recommended by Jacobson *et al* (123) is 0.1 mg. per kilogram of body weight daily for 4 days The white cell count should be determined 3 times a week during and for 2 weeks after the course of HN2, and complete blood studies, including hemoglobin, differential, and platelet counts, should be done at weekly intervals during this period. Other or more frequent hematologic examinations are carried out as indicated.

In our experience, a total of 0 4 mg per kilogram is generally a safe dose This may be given as 0 1 mg per kilogram daily for 4 days, 0.2 mg for 2 days or 0 4 mg. in a single dose. It is usually preferable to give the first course of treatment slowly, but once the patient's response is known, larger single doses may be given in subsequent courses (The toxicity of a large single dose, in our experience, is not appreciably greater than the same amount spread over several days and the period of discomfort to the patient is decreased) Blain and Nurnberger (24), however, suggest that the larger single doses of HN2 have been more toxic In patients with carcinoma of the lung and a normal blood picture, we have given 3 doses of 0 2 mg per kilogram with no serious toxic effects In patients with bone marrow involvement with leukemia, Hodgkin's disease, lymphosarcoma, or carcinoma, the drug should be given conservatively and the blood picture carefully followed

In 2 to 3 weeks, if the hematologic picture is not significantly altered or has already recovered, and the clinical response is inadequate, a second course of HN2 is indicated If the leukocyte count has not fully recovered but the clinical response is unsatisfactory, it is safer to give only 0 2 mg per kilogram If the clinical response is satisfactory, however, further treatment is usually withheld until there is evidence of relapse In some instances maintenance therapy has been attempted

INDICATIONS FOR USE

Hodgkin's Disease. The most useful clinical application of HN2 has been in the treatment of Hodgkin's disease (4,7,15b, 24,30,59,71, 94,95,96a,101,114,123,133,153a,196,208,224,227,229a,230,231,231b). The majority of patients, as high as 80 to 90 per cent in the larger series (4,7,59,94,95,101,123,133,230), have shown some benefit from a course of HN2 therapy. The improvement comes on rapidly following treatment. It may consist of a decrease in fever, relief of anorexia, and an increase in appetite, weight, strength, and feeling of well-being; these effects have been regarded as evidence of detoxification. Less frequently, pruritus and pain may be relieved. Objectively, there is a shrinkage of tumor tissue and its secondary manifestations, a decrease in size of an enlarged liver and spleen, and a rise in hemoglobin and serum proteins The remissions may last from 2 weeks to 1 year or longer, but 6 to 8 weeks is the average range. Occasionally, a complete remission does not follow the first course of treatment and several courses may be required to produce maximal improvement. Our usual procedure is to bring the leukocyte count down to 1,000 to 2,000 by careful manipulation of the dosage, since it is our impression that the longest and most satisfactory remissions are produced by the maximum safe dose The longer the remission produced by an adequate course of therapy, the better the prognosis for length of life and the response to subsequent courses of treatment

On histologic examination of the tumors following a course of HN2, Spitz (195) described enlargement of the reticulum and Sternberg-Reed cells, principally by fat; the nuclei were pyknotic or showed swelling with loss of chromatin pattern In the nodes containing these injured cells, intact dividing reticulum cells were also seen There was a decrease in the lymphocyte and eosinophil content in the nodes following HN2, and these cell types did not seem to reaccumulate to their original level.

In the vast majority of patients with Hodgkin's disease, the dissemination of the disease cannot be controlled, and x-ray or HN2 therapy, at best, can only produce a modest prolongation of life. Both x-rays and HN2 have a remarkable palliative action, which may keep the patient in relatively normal circumstances for the major part of the time during the natural course of the disease.

The clinician is interested in knowing which agent will give the more effective palliation in specific clinical situations.

In localized Hodgkin's disease, roentgenotherapy is the method of choice. It can be applied to the local areas of disease, and marked tumor regressions and prolonged clinical remissions are often induced. HN2 will also produce regressions in the disease and clinical improvement, but the remissions are shorter and may not be as complete as those induced by x-rays. Repeated remissions in relapsing patients, however, can be produced by HN2, and there is no evidence that the shorter HN2 remission adversely affects the course of the disease or its subsequent response to x-ray therapy.

HN2 appears to have its greatest usefulness in the treatment of widely disseminated Hodgkin's disease, in patients with systemic intoxication and in patients unresponsive to x-ray therapy. The use of HN2 may induce satisfactory general improvement and tumor regression, and x-rays may subsequently be employed, if indicated, to treat local or resistant manifestations of the disease. In the experience of Wintrobe and Huguley (229a), patients failing to obtain a remission from HN2 therapy were no longer responsive to irradiation. Indications for the use of HN2 may be illustrated by several clinical situations:

(*a*) A patient, in whom Hodgkin's disease was manifested by fever and symptoms of systemic intoxication, has been maintained in excellent condition for over 2 years, thus far, by single injections of 0.4 mg. of HN2 per kilogram whenever his fever began to return, which has been every 8 to 12 weeks

(*b*) Patients with enlarged livers, spleens, lymph nodes, pulmonary involvement, etc , in whom the disease was so extensive that localized x-ray therapy was not feasible, have sometimes shown remarkable regressions of the disease following HN2

(*c*) Patients with special complaints, such as pruritus, pain, generalized skin involvement, paraplegia, have been temporarily relieved by HN2 therapy. When the complaint is due to localized disease, such as a circumscribed bone lesion or infiltration of a vertebra, x-ray therapy may be more effective

(*d*) Some patients with generalized disease, with or without systemic intoxication, may appear to be refractory to x-ray therapy Some of these patients may show a gratifying response to HN2

therapy, and a trial of HN2 is probably justified in a patient who has been called "x-ray resistant." Wintrobe *et al.* (231) have described such a case.

Case 1. A. B., a 33 year old male, was well until August, 1944 when he noted a pleuritic type of pain in both sides of his chest and intermittent fever. In November, 1944 enlarged cervical nodes appeared. Biopsy of a cervical node led to a diagnosis of Hodgkin's disease and roentgenotherapy was given to the involved areas during February to July, 1945. At first, the nodes seemed to "melt away" under therapy, but later they became less responsive Fever, weight loss, and weakness progressed and nodes reappeared in the treated areas Roentgenotherapy seemed to have become ineffective

In August, 1945 the patient showed evidence of marked weight loss and the skin was deeply pigmented over his entire body. Temperature was 102 F There was a generalized peripheral adenopathy, but the liver and spleen were not palpable. The hematocrit was 36 mm., the leukocyte count, 8,200 Roentgenographic examination of the chest showed a mottled diffuse infiltration of the right lung field and some infiltration of the left, together with probable atelectasis of the lower lobe of the left lung.

The patient was given a total of 36 mg of HN2 (0 6 mg/Kg) from August 7 to 12. The white blood cell count fell to 650, the hematocrit to 20 mm., the platelet count to 50,000, and a few petechiae appeared in the skin. During this period he received 3,000 cc. of blood, and this raised his hematocrit to 45 mm. Subjectively, the patient improved, and on September 4, 1945 his temperature was normal, the nodes in his neck were no longer palpable, and the white blood cell and platelet counts had risen to 1,750 and 110,000, respectively Roentgenographic examination of the chest showed a decrease in the extent of involvement in both lungs

One month later, he was feeling well, had gained 30 pounds in weight and was afebrile, hematocrit, 45 mm, white blood cell count, 10,000 The signs of pulmonary involvement had regressed further The remission continued until November 16, 1945, when small cervical nodes appeared, a total of 0 2 mg of HN2 per kilogram of body weight caused these to regress.

In January, 1946, the cervical nodes again became enlarged and fever returned From January 25 to 29, he received a total of 30 mg HN2 (0 5 mg/Kg) The nodes decreased in size, the temperature became normal, and the patient felt well A few nodes persisted in the left posterior cervical triangle, although the patient felt well, in the later part of March he was given 0 1 mg of HN2 per kilogram, followed by irradiation (800 r.) to this area, with a reduction in the size of the nodes

On June 5, the nodes were slightly larger, fever had returned, and he had lost weight. From June 5 to 7, he received a total of 18 mg HN2 (0 3 mg/Kg), followed by irradiation (200 r) to the left infraclavicular region. The infiltration in the chest appeared to be increasing, and in July he received 400 r to the chest followed by 24 mg of HN2 (0 4 mg/Kg) during August 1 to 5.

A remarkable clinical improvement then occurred, he became afebrile, and gained 14 pounds in weight. On August 20 his hematocrit was 37 mm., and the leukocyte count 2,900.

He continued to feel well and to work until September, when fever returned and enlarged nodes appeared in the left groin. He was treated by irradiation (800 r.) to his left groin, and thereafter was given 24 mg. HN2 (0.4 mg./Kg.) between September 10 and 14. Again, there was considerable improvement, with gain in appetite and weight and disappearance of fever. This remission lasted about a month; then fever returned On November 11 he was readmitted to the hospital because of severe pruritus, cough, chills and fever, and loss of weight The hematocrit was 33.5 mm., the white blood cell count 6,250 Roentgenograms of the chest showed a new infiltration of the right apex and increased infiltration in the left midlung field. He was given 600 r. of spray irradiation, a total of 18 mg HN2 (0 3 mg./Kg.), blood transfusions, and urethane, without any effect on the fever or pruritus. Hematocrit declined to 16 2 mm., the leukocyte count to 450, and the platelets to 20,000 He died on December 5, 1946, about 16 months after the start of HN2 therapy

(e) A course of HN2 may be considered as a therapeutic diagnostic procedure in patients with unexplained fever, in whom lymphoma is suspected. A prompt and prolonged fall in the fever would be indicative of a lymphomatous process for, in our experience, HN2 has not exerted a favorable influence on infectious diseases

The ability of HN2 to induce remissions in "x-ray fast" Hodgkin's disease and, far less commonly, in lymphosarcoma (230) impels speculation As previously noted, we are not prepared to believe that there is a dissociation in the response of Hodgkin's disease tissue to HN2 and to x-rays, and we must seek for some other explanation In one patient with generalized Hodgkin's disease, an enlarged spleen, fever, and anemia, roentgenotherapy to a group of enlarging inguinal nodes induced a complete remission, including a rise in hemoglobin and a reduction in spleen size, for 5 months This remission was similar to, but of longer duration than, that induced previously by HN2 therapy In generalized disease with systemic intoxication it is sometimes difficult to locate the active focus of disease, and it seems reasonable to expect that a systemic form of therapy, such as HN2, would ferret out this area It follows, of course, that if x-rays had been properly applied a similar therapeutic result would have been obtained Also, some of the enlarged nodes in Hodgkin's disease, which appear to be "x-ray resistant"

may possibly represent secondary reactions to an active focus of disease elsewhere. If this focus were controlled, the nodes might regress spontaneously. If x-rays were coincidentally applied, the regression of the nodes might be interpreted as an indication that they had become sensitive to x-rays again It is apparent that an understanding of the biology of Hodgkin's disease would clarify these problems

Lymphosarcoma. In this category is included a number of allied diseases: lymphocytic and lymphoblastic sarcoma, lympholeukosarcoma, reticulum cell sarcoma, and giant follicle lymphoma. The prognosis in these various conditions differs considerably, but their response to HN2 therapy is related to the rapidity with which the disease is progressing and the stage when therapy is begun (7,30, 59,71,94,95,123,133,171,196,208,224,229a,230,231)

In the early stages of slowly progressive disease prolonged remissions consisting of complete regressions of enlarged nodes have been induced by HN2; these have continued for longer than one year When the nodes recurred, another course of HN2 was effective It is apparent that these patients will also respond satisfactorily to roentgenotherapy

Occasionally, slowly progressing lymphosarcomas, particularly of the reticulum cell type, are extremely resistant to locally applied x-rays These tumors are also relatively unaffected by HN2 therapy Large doses of HN2 may induce a transient and slight decrease in the size of the nodes, but recurrence is rapid A case of this type has been described by Goodman *et al* (94,95)

Case 2 E W, a 42 year old woman, was first seen in September, 1943, when she complained of severe pruritus, substernal *pain*, cough, and dyspnea Study disclosed a rapidly growing mediastinal lymphosarcoma, verified by biopsy of a cervical node. Radiation therapy gave temporary relief but finally failed to retard the growth of the mediastinal mass Findings in February, 1944, included fever, generalized severe pruritus, cough, dyspnea, bilateral pleural effusion, enlarged cervical nodes, and an intrathoracic mass. The blood picture revealed no great abnormality She was given a total of 0.5 mg. of HN2 per kilogram of body weight over a period of 9 days There was no objective or subjective improvement, the ingravescent course progressed, and death occurred 5 weeks after HN2 therapy Postmortem examination verified the diagnosis of lymphosarcoma, which was found to involve the lungs, pleura, pericardium, myocardium, diaphragm, and ribs

In another category are the extremely aggressive forms of lym-

phosarcoma, which often appear acutely and disseminate rapidly. The tumors are radiosensitive, but they recur in a few weeks or new ones crop up elsewhere. It is soon apparent that roentgenotherapy cannot keep the disease under control. The use of HN2 in these patients has often induced remarkable regressions in lymph nodes and other involved areas, and definite symptomatic improvement, but within a few days or weeks the disease has recurred and continued on its rapid course. Further courses of HN2 prove even less effective, and the bone marrow often appears to be unusually susceptible, so that HN2 should be given cautiously. An example of an extremely aggressive type of lymphosarcoma, only slightly affected by repeated courses of HN2 which finally produced severe bone marrow injury, is shown in the following case report (133).

Case 3 B. M., a 37 year old man, first noted an enlarged submental node in July, 1944. In the next few months a generalized lymphadenopathy appeared, these nodes were effectively controlled with x-rays In January, 1945, the nodes recurred On examination he was afebrile, liver and spleen were not palpable, and roentgenogram of the chest appeared normal. The hemoglobin was 13.7 Gm, the white blood cell count 5,000 Between February 5 and 13 he was given a total of 28 mg of HN2 (0 42 mg/Kg.) A severe leukopenia developed, and some regression in the enlarged nodes

Three weeks later the patient complained of epigastric pain, the cervical nodes were enlarging, and there was a 6 pound loss of weight. The hemoglobin was 12 2 Gm, the white blood cell count 2,200 From March 12 to 19 a total of 28 mg of HN2 (0 44 mg/Kg) was given The enlarged cervical nodes regressed strikingly, and there was considerable clinical improvement.

By the end of another 3 weeks the cervical nodes were again enlarging, the hemoglobin was down to 9 4 Gm., and the white blood cell count to 2,000. He was given 3,000 cc. of red cells, and a third course of HN2 from April 18 to 21, again a total of 28 mg (0 43 mg/Kg). Once more a striking regression enlarged cervical nodes occurred, and the patient felt somewhat better. The white blood cell count had fallen to 950

Within 1 week, the cervical nodes were again enlarging. Roentgenotherapy had little effect. The hemoglobin had in the meanwhile fallen to 7.0 Gm, and he was given 3,000 cc of red cells During May 21 to 24 he received the fourth and last course of HN2, a total of 28 mg (0.43 mg/Kg) The cervical nodes became smaller and he immediately felt better. Less than 1 week after completion of treatment, soreness in the chest developed, with bleeding from nose, gums, and rectum, as well as purpura and hematuria The white blood cell count remained below 1,000, the platelets fell to less than 100,000, and the hemoglobin dropped precipitously from 9 4 to 3 4 Gm The patient died on June 6, 1945, 11 months after onset of his disease and 4 months after initiation of HN2 therapy

At autopsy, the following diagnoses were made: lymphosarcoma involving the cervical, axillary, mediastinal, and retroperitoneal nodes; diffuse hemorrhage involving most of the tissues; microscopic evidence of degenerative nuclear changes in the neoplastic cells.

Patients with far-advanced lymphosarcoma, who have failed to respond to adequate roentgenotherapy, rarely show a substantial response to HN2. In some instances, transient regression of enlarged nodes and symptomatic improvement may occur, but the patient quickly relapses. Nevertheless, HN2 may sometimes be of some real benefit in an apparent terminal case. Occasionally, the disease is so extensive that irradiation is not feasible, and HN2 may induce remissions of 1 to 2 months Patients have responded satisfactorily to roentgenotherapy, and then returned many months later in serious condition with generalized disease. A systemic form of therapy may rapidly improve the condition of these cases. A course of HN2 should be spread over a week or more in such patients. We have seen patients in whom the tumors were very sensitive but, although rapid regression of the tumors occurred following a course of HN2, the patients declined rapidly and died Such a case is described by Osborne *et al.* (171).

Case 4. A. P, a 61 year old man, was admitted to the hospital on October 15, 1946 in a comatose condition. On examination he was jaundiced, and large firm nodes were found in the anterior cervical region bilaterally and in both inguinofemoral regions A mass was palpated in the right lower quadrant of the abdomen, and an ill-defined mass was felt in the left upper quadrant There was edema of the right leg associated with multiple indurated bluish nodules involving the skin and subcutaneous tissue of the right leg below the knee Some of the lesions were elevated about 2 cm above the normal skin Many smaller cutaneous and subcutaneous nodules were present in the area extending two-thirds up the thigh The liver and spleen were not enlarged The white blood cell count was 4,650 Biopsy specimens taken from the skin and a lymph node revealed the presence of lymphosarcoma Although the patient appeared to be moribund, it was decided to give him a course of nitrogen mustard therapy—0 1 mg per kilogram of body weight daily for 4 days Two days after the treatment the patient was mentally alert and the jaundice appeared to be subsiding The lesions on the right leg became soft and continued to involute, until on the tenth day there was only residual pigmentation remaining in the skin. The abdominal masses and the enlarged lymph nodes decreased in size *The white blood cell count continued to fall in spite of multiple blood transfusions*; on the seventh day after treatment it was 1,000 and on the tenth day, 850 Pneumonia developed and the patient died on the twelfth day after treatment. Autopsy showed lobar pneumonia with fibrinous pleurisy

and cardiac dilatation. There was no gross or microscopic evidence of lymphosarcoma in the lymph nodes, viscera, and skin

In instances in which lymphosarcoma in the neck or thorax has produced difficulty in breathing, HN2 may give rapid and dramatic relief, and its use actually may be regarded as an emergency procedure. Craver (59) has described a case in this category; an elderly woman with lymphosarcoma growing diffusely in the side of the neck, fixed to the larynx and trachea, and extending through the region of the thyroid gland and down through the upper thoracic aperture She was orthopneic and had marked stridorous respirations. The surgeon believed tracheostomy would be useless, as he would have to cut through about an inch of tumor overlying the trachea and it was evident that there would continue to be obstruction well below the level of the operation. X-ray therapy was thought to be useless and probably dangerous; a large dose, to get a quick effect, would probably cause complete obstruction of the airway, and fractionated doses would act so slowly that it was believed that death would occur before the obstruction could be relieved A single injection of HN2, 0.2 mg. per kilogram, caused rapid and remarkable relief and, within 24 hours, the patient was able to breathe more comfortably The course of HN2 was completed and the patient then received x-ray treatment over the neck without complications

Histologic changes have been described in lymphosarcomas treated with HN2 (119,195) Spitz (195) has studied the problem in detail. In lymphocytic lymphosarcoma, no definite cytologic changes are seen, but the lymphocytes disappear following HN2, and the residual stroma of the tumor is unveiled In reticulum cell sarcoma, ballooning of the cytoplasm by fat and extensive nuclear fragmentation have been observed While these degenerative changes, which decrease the number of cells in the tumor, are in progress, unaltered cells in active mitosis may be found in the same node

If x-ray therapy is available, HN2 does not appear to have an important place in the treatment of lymphosarcoma Tumors not responding to x-ray therapy ordinarily do poorly on HN2 Occasionally, however, HN2 may prove to be of great value, and the therapeutic possibilities of HN2 should be kept in mind in the management of lymphosarcomas

Mycosis Fungoides. The use of HN2 has been reported in 21 patients with mycosis fungoides (59,106,107,133,137,171,179,208,209, 224). The nature of mycosis fungoides is unclear, but there is no doubt that what has been called mycosis fungoides often terminates as some type of lymphoma. The clinical responses obtained in patients with mycosis fungoides, consequently, may be related to the rate of progress of the disease, the stage of the disease when treatment is initiated and the underlying neoplastic condition.

Some of the patients with slowly progressive, extensive chronic mycosis fungoides have failed to show a satisfactory response to HN2 (133). In most cases, however, a rapid and marked therapeutic effect has been obtained, which has lasted for 3 to 5 months or longer. The severe pruritus is relieved, the skin lesions become pale and regress, and the patient shows striking symptomatic improvement This improvement may occur in patients unsuitable for x-ray therapy (107) One of the cases reported by Osborne *et al* (171) illustrates a response of this type.

Case 5. G. S., a 54 year old white woman, gave a history of a rash beginning on the face in May, 1944, following a severe sunburn The rash spread to involve most of the body, accompanied by severe itching The condition did not improve and, in August, 1946, the patient noted a number of tumor masses appearing in her skin

On October 17, 1946, she revealed a confluent erythematosquamous eruption with sharply demarcated borders, involving the scalp, face, forearms, and outer surfaces of the upper portions of the arms, and the upper part of the chest and back A similar eruption was also present on the thighs and lower parts of the legs Associated with this eruption were multiple plaquelike tumors on the thighs, legs, face, and neck Several of the larger lesions showed an early tendency toward ulceration A complete physical examination revealed no enlargement of lymph nodes, liver or spleen, or other involvement of the viscera A clinical diagnosis of mycosis fungoides was made A biopsy specimen removed from one of the tumors on the neck disclosed pathologic changes consistent with the diagnosis of mycosis fungoides The blood count was within normal limits Roentgenographic examination of the chest showed a calcified node in the right hilar region and a slight generalized fibrosis in the pulmonary fields From November 5 to 8 the patient was given a total of 0 4 mg HN2 per kilogram of body weight A moderate degree of anemia and leukopenia developed, which disappeared in 4 weeks

Immediately after the completion of treatment, the skin improved considerably, with diminution in the amount of redness and scaling and a definite decrease in the size of all nodules The itching had almost entirely disappeared By the sixth day after treatment, the patient was subjectively much im

proved On examination on December 5, approximately one month after treatment, most of the nodules had regressed completely and only a few could be palpated The diffuse erythroderma had improved at least 90 to 95 per cent, leaving some residual atrophy and telangiectasia. Examination on January 23, 1947, revealed two active nodules in the right malar region and one involving the right flank, but no other evidence of recurrence. About 6 months after the initial course of HN2, the patient had begun to lose weight, a mass appeared on her right arm, the itching had returned and she became progressively worse

In rapidly advancing mycosis fungoides with large and extensive skin tumors and internal involvement, HN2 has caused remarkable improvement, but the therapeutic result is transient, and the patient may relapse in a few days Henstell *et al.* (107) have described a patient who responded briefly and then became resistant to HN2 therapy.

Case 6. K S., a 50 year old man, developed an itching rash on the right leg in 1944 He was treated by various methods, including x-rays, with brief periods of improvement From October, 1945, he had been given large amounts of irradiation and arsenic, with little effect.

On January 18, 1947, he was admitted to the hospital for study and treatment Except for extensive lesions of the skin, physical examination revealed nothing abnormal The trunk and extremities were covered with numerous annular, serpiginous, infiltrated lesions tending to clear in the center and spread peripherally Some involved fairly large plaques of skin, without central clearing, and with tendencies to coalesce On the left hand, in the upper right groin, and on the feet extensive sloughing of the skin had taken place, leaving large areas of denudation and deep ulcers These lesions were intensely pruritic A mild anemia was present A skin biopsy confirmed the diagnosis of mycosis fungoides

From January 12 to 27, 1947, he received a total of 42 mg of HN2 By the fifth day after the beginning of treatment there was a marked decrease in the number and size of the lesions and in the intensity of itching, and upon completion of the course of therapy the extensive lesions had almost completely disappeared The denudations of the left foot and right thigh had healed completely The right foot, which was the most extensively involved, showed considerable but not complete healing New areas of skin began to appear and the secondary infection cleared without other therapy Two weeks later, however, reactivation of the disease was evidenced by a dozen or so tiny new lesions and an increase in exudation over the pre existing areas By February 26, the new lesions had become fairly large and all lesions were oozing considerably

From February 26 to March 4 he received a total of 48 mg of HN2 By March 2 the lesions began to regress and by March 10 about half the lesions had disappeared and the remainder had regressed to at least one half their original size This improvement was short lived, and in a few days an increase

in oozing appeared By April 10, 1947, his entire body was covered with circinate, crusted lesions, and the epithelium of both feet and hands was extensively denuded

From April 11 to 21, 1947, he received a total of 42 mg of HN2; 0 6 mg of colchicine was also given 3 times a day for 1 week in the hope that it would augment the effects of HN2, but was then discontinued because of severe nausea, vomiting, and diarrhea. On April 19, total body irradiation, 30 r. to each side, was begun twice weekly. On May 16 the white blood cell count had dropped to 600. The skin lesions continued to ulcerate, the general condition deteriorated and the patient died on June 4, 1947.

Microscopically, the skin lesions of mycosis fungoides may show remarkable alterations after a course of HN2 Henstell *et al.* (107) have suggested that those lesions containing a large number of reticulo-endothelial cells respond most satisfactorily to HN2

Chronic Lymphatic Leukemia. The response of chronic lymphatic leukemia to HN2 is less predictable than that of chronic myelogenous leukemia (45,47a,59,94,95,123,133,213,224,227,230,231). In the early or relatively asymptomatic stage of the disease, HN2 may cause a reduction in the elevated white count and the size of the enlarged lymph nodes and spleen, a rise in the hemoglobin level, and possibly some increase in the feeling of well-being This improvement may continue for many months, Jacobson *et al* (123) have obtained a remission of longer than 21 months It is likely that these responsive patients would have also been benefited by other forms of therapy, such as x-rays, radioactive phosphorus, and urethane Some of these patients may occasionally be adversely affected by HN2, it should consequently be given cautiously, perhaps at doses of 0 1 mg per kilogram of body weight every other day, with repeated examinations of the blood for indications as to further dosage.

In far-advanced disease, with hepatomegaly and splenomegaly, enlarged nodes, and severe anemia, HN2 therapy has been almost uniformly unsuccessful There may be an appreciable reduction in the size of enlarged nodes, but a rise in hemoglobin does not occur, bleeding tendencies may become more severe, and the patient is not improved (47a,133). In one series of 6 patients with far-advanced disease 5 were dead within 3 months after HN2 therapy was started, and the sixth patient, whose history is given below, survived for 9 months, but did not experience any real benefit

Case 7. J. S., a 53 year old man, complained of a sore throat in April, 1943 On examination enlarged tonsils and a generalized peripheral adenopathy were present but the liver and spleen were not palpable Hemoglobin was 11 Gm, white blood cell count 36,400, with 7 per cent polymorphonuclears and 93 per cent lymphocytes; the sternal marrow showed a differential count of 3 per cent polymorphonuclears, 92 per cent lymphocytes, 2 per cent metamyelocytes, 2 per cent myelocytes, 1 per cent hemocytoblasts, and 3 early and 5 late nucleated red cells per 100 white cells X-ray therapy caused the enlarged organs to regress, and the patient felt well and continued to work until August, 1944 He complained of weakness and anemia, and by September, 1944, the hemoglobin had fallen to 6 3 Gm., the peripheral nodes were increasing in size, and the spleen and liver were palpable In November, 1944, hemoglobin was 3 2 Gm, and white blood cell count 129,000. He was treated with multiple blood transfusions, and in January, 1945, he was admitted for HN2 therapy. On examination, the peripheral nodes and tonsils were enlarged and the liver and spleen were down 2 fingerbreadths below the costal margin. Hemoglobin was 7.8 Gm, red blood cell count 2,400,000, and white blood cell count 60,000, with 1 per cent polymorphonuclears and 99 per cent lymphocytes The patient was transfused with 1500 cc. of blood and, from January 26 to February 2, 1945, was given a total of 36 mg. of HN2 (0 41 mg./Kg) The white blood cell count fell slightly but the anemia became more severe and he obtained no benefit from the treatment

On March 10, 1945, the hemoglobin was 3 9 Gm., and he was transfused up to 12 5 Gm with 500 cc. of whole blood and 6,000 cc. of red cells From March 13 to 19, 1945, he received a total of 36 mg of HN2 (0 41 mg /Kg.). The white blood cell count fell from 90,000 to 10,000 and his lymph nodes regressed moderately, but he showed no clinical improvement Ten days after this course of HN2, transfusions were again started, these raised the hemoglobin to 15 Gm, and he felt much stronger

About 3 weeks later, on May 10, the hemoglobin had fallen to 7 6 Gm, and the right tonsil was enlarging 300 r. of x rays caused the tonsil to regress, and 2,000 cc of red cells raised the hemoglobin to 9 4 Gm From May 16 to 19, a third course of HN2 was given, a total of 32 mg (0 39 mg /Kg) This resulted in a severe and persistent leukopenia of less than 2,000 The hemoglobin fell rapidly, during the next month, he was given 2,000 cc of red cells, and 3,500 cc of whole blood, with slight benefit

On August 6, 1945, the hemoglobin was 2 7 Gm, he was transfused with 2,300 cc of red cells, which raised the hemoglobin to 10 5 Gm The anemia promptly recurred, petechiae developed over the body, the peripheral adenopathy increased, and he died on October 9, 1945

An occasional patient with far-advanced lymphatic leukemia may show some improvement associated with blood transfusions and HN2 therapy. It is difficult to anticipate the patients who will respond, or to attribute the favorable response directly to the use of HN2

Chronic Myelogenous Leukemia. HN2 has been useful in inducing clinical remissions in chronic myelogenous leukemia (45,47a, 59,94,95,123,133,213,224,227,229a,230,231). These remissions consist of a fall in the white blood cell count, a reversion in the differential cell count toward normal, a rise in hemoglobin, and a reduction in the size of an enlarged spleen. These objective evidences of improvement are associated with symptomatic relief and an increase in strength and well-being. A dosage of 0.3 to 0.6 mg. or more of HN2 per kilogram of body weight may be required to induce a remission. The drug is given cautiously and it is continued until the white cell count has been brought down to about 10,000 The remissions usually last from 1 to 4 months, with an average of about 2 months In patients in relatively good condition, clinical remissions can be repeatedly produced An example of a satisfactory response is shown in the following case report (94,95).

Case 8. B C, a 52 year old man, had obtained satisfactory remissions from x-ray treatment applied at approximately yearly intervals from 1941 to 1944 In May, 1945, fatigability returned, the leukocyte count was 293,000, and the red blood cell count was 3,110,000 The spleen extended 3 cm. below the umbilicus and the liver 5 cm below the costal margin The patient was given a total of 0.5 mg of HN2 per kilogram of body weight over a 2 week period, and a 1,000 cc. transfusion of blood Six weeks later the hematocrit was up to 50 mm, and the white blood cell count was 25,800 The spleen and liver had decreased appreciably in size The patient had gained 10 pounds in weight and was able to do light work Four months after the start of therapy the patient was still feeling well, was doing regular work, and maintaining the gain in weight but, because of a slight anemia (hematocrit, 40 mm) and a leukocyte count of 50,400, he was hospitalized and given 0 4 mg HN2 per kilogram of body weight Following this second course of therapy he felt well, the hematocrit again rose, and the white blood cell count decreased to 11,300 Two months later he was found to be slightly anemic and the leukocyte count had risen to 103,000. He was given 0 3 mg HN2 per kilogram body weight, and an increased hematocrit and a decreased leukocyte count was again obtained Nine months after the first course of therapy, the patient was in a fair state of remission, both hematologically and clinically

Many of the patients with myelogenous leukemia, who have been reported in the literature because they received HN2, were in the late stages of the disease, in poor condition, and no longer responding to x-ray therapy HN2 lowered the leukocyte count in some of these patients, but a spontaneous rise in hemoglobin did not occur, bleeding, if present, usually continued, and there was no general

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Bleeding from nose and gums began, the hemoglobin level fell precipitously, the temperature rose, and she died on January 23, 1946, about 22 months after the onset of the disease and 5 weeks after the beginning of HN2 therapy During this 5 week period she had received the equivalent of about 2 0 mg of HN2 per kilogram body weight without any striking effect on the "blast" cells in the peripheral blood.

Treatment is indicated in chronic myelogenous leukemia if symptomatic complaints are present The clinician now has a variety of therapeutic agents from which to choose, such as Fowler's solution, urethane, radioactive phosphorus, nitrogen mustard, and x-rays. These agents will all prove effective in early and responsive cases; they practically always fail in the terminal cases In the early cases, irradiation of the spleen usually gives a satisfactory and prolonged response An adequate course of HN2 may give an equally satisfactory remission, but it is probably of shorter duration The physician may also use Fowler's solution, urethane, or radioactive phosphorus with similar therapeutic results

After a clinical remission has been induced with HN2, it is usually our practice to wait until evidence of relapse appears before another course is instituted Later, if the disease appears to be fairly active, maintenance therapy, based on periodic doses of HN2, may prove satisfactory for long periods (230) The dosage is usually in the range of 0 2 mg per kilogram of body weight every 3 to 8 weeks, and the blood picture must be carefully watched for proper dosage and frequency of administration.

If the spleen is greatly enlarged, or the disease has infiltrated a circumscribed area, locally applied x-rays will prove more effective Leukemia cutis has not shown a satisfactory response to HN2 in our experience

It is too early to determine whether HN2 prolongs life in leukemia or whether, conversely, it increases the incidence of complications or hastens the onset of acute leukemia, as compared with the other types of therapy. From the available evidence, it seems probable that if any alteration is produced by HN2 in the course of the disease, it will be a minor one (47a)

Acute Leukemia The effects of HN2 in acute and subacute forms of myelogenous, lymphatic, and monocytic leukemia have been largely unsatisfactory. Jacobson *et al* (123) treated 2 patients without benefit. Wintrobe *et al* (230), in a group of 8 patients, ob-

clinical improvement. It is probable, however, that the use of HN2 and blood transfusions may make the patients more comfortable and briefly prolong life The extraordinary resistance of the white blood cell count to nitrogen mustard, and the failure of large doses of nitrogen mustard to induce clinical improvement in an adult in the terminal stage of leukemia is shown in the following case report.

Case 9. A. F., a 29 year old woman, had an episode of prolonged vaginal bleeding in Mar., 1944. During the laparotomy for removal of an ovarian tumor, an enlarged spleen was discovered and removed. Leukemia was diagnosed after microscopic examination. The patient remained fairly well for 15 months and received occasional injections of liver extracts. In Aug, 1945, she entered Memorial Hospital complaining of weakness, dizziness, occasional headaches, and a tendency to bruise easily. On physical examination she appeared to be in good condition; lymph nodes and the liver were not enlarged. Hemoglobin was 9.4 Gm , leukocyte count, 113,000, with 44 per cent polymorphonuclears, 18 per cent metamyelocytes, 32 per cent myelocytes, and 5 per cent lymphocytes The platelet count was 79,000, and bleeding and clotting times were normal

As part of a special study, she was treated with 1 Gm of thiouracil daily, beginning on September 18, 1945, and continuing for about 10 weeks This treatment was without benefit; the leukocyte count continued to rise, and the complaints of weakness, headaches, and ecchymoses became more prominent On December 6, 1945, the patient began to vomit continuously, and a pain developed in the right upper quadrant She was admitted to the hospital 4 days later in poor condition; there was a slight fever, the peripheral lymph nodes were moderately enlarged, and the liver was palpable 10 cm below the costal margin. The eyegrounds showed blurring of the disks with old and new retinal hemorrhages Hemoglobin was 10 0 Gm , leukocyte count, 441,000, with 14 per cent polymorphonuclears, 12 per cent metamyelocytes, 37 per cent myelocytes, 4 per cent eosinophilic myelocytes, 4 per cent premyelocytes, 17 per cent "blasts," and 13 per cent nucleated red cells From December 13 to 20, she was given a total of 39 mg of *tris*(2-chloroethyl)amine hydrochloride (0.62 mg/Kg) Symptoms became worse during the first few days of the treatment, but then patient rapidly improved By December 27, the leukocyte count had gradually fallen to 60,000, the headaches and blurred disks had cleared, the liver was smaller, and she felt generally much improved

Three days later, however, the white cell count began to rise, headaches recurred, and patient complained of cramps in the legs She was consequently started on nitrogen mustard therapy, and for purposes of comparison, HN2 was used From January 2 to 10, 1946, she was given a total of 39 mg. of HN2 (0 65 mg./Kg). The leukocyte count fell slightly, but there was no real symptomatic relief. On January 13, the white cell count began to rise again, and the blood smear showed almost 100 per cent "blast" forms During the next 2 days she was given a total of 20 mg of HN2 (0.35 mg/Kg) without avail

to the many other methods in use for treating this chronic disorder is an important problem for further clinical investigation.

Carcinoma of Lung. The results obtained from the use of HN2 in 102 patients with primary carcinoma of the lung have been reported (24,42,59,131,133,191,209,224). The dosage of HN2 has varied; Skinner *et al* (191) have used 0 4 mg per kilogram in a course of treatment, and Boyland *et al.* (42) and Karnofsky *et al.* (131) gave doses of 0 6 to 0 8 mg. per kilogram in a single course Our schedule has usually been 0 2 mg. per kilogram as a single dose daily for 2 days. If the white cell count is not significantly depressed in 1 week, a third dose of 0 2 mg per kilogram is given, and if the clinical response is unsatisfactory and the white cell count permits, a fourth dose is given the following week Boyland *et al* state that patients may tolerate 0 2 mg. per kilogram weekly for 10 weeks

A single course of HN2 induced symptomatic improvement, for periods of 2 to 12 weeks (average, 5 weeks) in the majority of patients Subsequent courses were not as effective and only in a few instances did the use of HN2 appear to prolong life for several months. The symptomatic relief usually appeared within 1 week after start of therapy About 60 to 65 per cent of the patients experienced a reduction in pain, cough, and dyspnea, and an increase in strength, appetite and well-being Hoarseness due to paralysis of the recurrent laryngeal nerve was not relieved (42) The agent appeared to loosen the cough, and increased sputum temporarily before it diminished, with concomitant decrease of the cough. Deep chest pain was frequently relieved The improvement in general well-being was associated with an increase in strength, appetite, and activity Symptoms related to obstruction of the superior vena cava were improved in 8 out of 11 cases.

About 40 to 50 per cent of the patients show objective evidence of improvement, including a decrease in pulmonary densities and increased aeration of the lung, regression of metastases, decrease in effusion, decrease in elevated venous pressure and, in one case, a remission in neurologic signs

The objective and subjective response of patients with a rapidly growing carcinoma was usually more dramatic but of shorter duration The therapeutic effects of HN2 in a patient with a rapidly ad-

served "poor" results in 5 and "fair" results in 3 The improvement was temporary and unsustained by further therapy. Two patients obtained relief from severe bone pain with small doses of HN2. Burchenal (45) treated 19 cases of acute leukemia with SK 136, a nitrogen mustard derivative. Since the clinical effects produced by SK 136 are similar to those from HN2, his results are considered here A fall in the leukocyte count occurred in all of the patients, and 11 showed some subjective improvement lasting 2 weeks to 3 months In 3 patients a brief remission occurred, associated with a rise in hemoglobin, a return of the differential white blood cell count toward normal, and general clinical improvement It was thought likely that these patients represented spontaneous remissions coincident with nitrogen mustard therapy Urteaga *et al* (214) obtained no clinical improvement from HN2 in 6 patients with acute leukemia.

It is apparent that HN2 is of little value in the treatment of acute leukemia, but it may occasionally produce some temporary symptomatic relief and, possibly, in conjunction with blood transfusions, cause a slight prolongation of life.

Polycythemia Vera. A total of 13 patients with polycythemia vera, treated with HN2, have been reported in the literature (31, 71,123,224,227). Jacobson *et al* (123) noted prompt symptomatic improvement in all of 5 patients treated, the remissions lasting 3 to 17 months Wilkinson and Fletcher (227) observed transient symptomatic improvement in 2 of 3 patients, and a satisfactory clinical remission in one of these patients Excellent clinical remissions of 3 to 5 months and a reduction of the spleen size were obtained in the 4 cases treated by Faloon and Gorham (71)

HN2, therefore, is another agent that may be added to the list of compounds influencing polycythemia vera It has not been in use long enough to determine consistency of therapeutic activity, duration of remissions, incidence of untoward complications, and advantages over other forms of therapy In comparison with radioactive phosphorus, which is regarded by many as the treatment of choice in polycythemia vera, HN2 produces nausea and vomiting during its use, and the remissions may be shorter On the other hand, it is easier to use, acts more rapidly in producing symptomatic relief, and the dosage can be controlled more safely The relation of HN2

hemoptysis, pain in his right chest, weakness, anorexia, and weight loss developed. Roentgenographic examination of the chest showed a right infraclavicular, intrathoracic tumor, and patchy consolidation of the right lower lobe. Bronchoscopy disclosed a mass occluding about one-half of the right main bronchus; a biopsy of this mass established the diagnosis. The lung tumor increased in size, cervical nodes became palpable, and in February, 1942, HN2 therapy was begun. At this time the hemoglobin was 13 Gm., the white blood cell count, 13,100 Between February 23 to 28, 1946, he was given a total of 32.5 mg HN2, in 6 doses of 0.1 mg. per kilogram Marked subjective improvement, with decrease in dyspnea, cough, and cessation of hemoptysis, increase in appetite, and weight gain of 5 pounds occurred. By 19 days after treatment the white blood cell count had fallen to 5,300. A roentgenogram of his chest 6 weeks after treatment showed good clearing of the infiltrative changes in the right lower lobe, but the density in the right upper lobe was unchanged. The symptomatic remission lasted about 8 months

In October, 1946, dyspnea and hemoptysis recurred, and a roentgenogram of the chest showed atelectasis in the upper third of the right lung From October 29 to November 1, 1946, he received a total of 25 mg. HN2 (0 5 mg./Kg.) in the Outpatient Department There was a transient period of nausea, weakness, and weight loss due to the HN2, followed by symptomatic improvement. The roentgenograms of the chest remained unchanged

During the next 8 months he was ambulatory, but he gradually became weaker and more dyspneic, and weight loss was progressive. In July, 1947, roentgenograms of his chest showed a complete atelectasis of the right lung with a mediastinal shift to the right. Hemoglobin was 10 Gm., white blood cell count, 27,000; and he was running a low grade fever which subsided with penicillin therapy From July 10 to 14, 1947, he received a total of 25 5 mg. HN2 in 3 injections of 0 2 mg per kilogram body weight The white blood cell count fell to 2,300 and the hemoglobin to 7.4 Gm within 3 weeks Dyspnea improved slightly, but the general change in his condition was insignificant He died 1 month later, in August, 1947

The indications for the use of HN2 in primary lung cancer have been tentatively suggested (131) Inoperable, but localized and relatively asymptomatic, carcinoma of the lung is best treated by x-rays. In widespread, but slowly growing, tumors producing severe symptoms, HN2 may be useful in the management of the patient. It may occasionally induce prolonged remissions when used alone; in most cases, however, it may be given in an attempt to induce symptomatic relief, and x-ray therapy can be used, if necessary, to control localized and troublesome manifestations of the disease

Rapidly advancing lung cancers usually do not respond satisfactorily to x-ray therapy, and furthermore, its use is considered hazardous in patients with obstruction of the superior vena cava In

vancing carcinoma of the lung (anaplastic) is illustrated in the following case report (131).

Case 10. L. C, a 60 year old man, noted anorexia, weakness, and weight loss in October, 1945, followed by cough and hemoptysis On examination in January, 1946, he was moderately obese; he complained of weakness and dyspnea. He was bedridden, orthopneic, and in poor condition. There was a mass of enlarged nodes in the left supraclavicular area Roentgenographic examination of the chest showed enlargement of the left hilum and a circular density in the left midlung field Aeration of the left lower lung field was impaired. Pathologic diagnosis was made on a biopsy of a supraclavicular node.

From January 24 to 29 he received a total of 40 mg of HN2 (0.5 mg/Kg). Within 2 days, dyspnea and general condition improved, hemoptysis decreased, and he became ambulatory The supraclavicular mass decreased markedly in size; on February 5 a roentgenogram of the chest showed a slight decrease in the left hilar prominence, and on February 20 a slight decrease in the density of the midlung field and improved aeration of the left lower lung field was noted Seventeen days after start of treatment, the white blood cell count was 1,200 and hemoglobin had fallen to 8 Gm. The clinical remission lasted for 3 weeks

On February 15, he began to relapse and complained of slight hemoptysis, weakness, and chest pain On February 24, the white blood cell count was 4,000, the hemoglobin, 8 Gm. On February 26, he was given 16 mg. of HN2 (0 2 mg./Kg) in a single injection In 7 days the white blood cell count fell to 1,800, and the hemoglobin to 7.1 Gm He was much improved symptomatically for 2 weeks; then, because of beginning relapse, a course of 3 injections of 0.1 mg of HN2 per kilogram (25 mg. total) was given. This resulted in another 2 week period of symptomatic improvement

On March 30, enlargement of the liver was noted He was given a fourth course of HN2 totalling 38 mg (0 5 mg/Kg.), and 1200 r of x-rays to the enlarged nodes in the left supraclavicular area These nodes regressed slightly but no clinical improvement occurred A leukopenia of 1,200 developed, and the hemoglobin fell to 6 7 Gm in spite of a 300 cc blood transfusion. He continued downhill, and died on April 19, 1946

At autopsy, a bronchogenic carcinoma arising in the bronchus of the left upper lobe with metastases to the liver, left kidney, left adrenal, right lower lung, pancreas, spleen, peribronchial, peripancreatic and periportal lymph nodes was found

The beneficial effects in the more slowly advancing disease take longer to develop, and the remissions, also, are usually longer. The palliative effects of HN2 in a patient with a relatively slow-growing bronchogenic carcinoma (Grade III) is shown in the following case report (131)

Case 11 G P, a 65 year old man In November, 1945, a productive cough,

ulcerated lesion protruding into the lumen of the lower esophagus. Biopsy of the esophageal lesion and the left supraclavicular node led to the diagnosis of adenocarcinoma

The tumor was clearly inoperable, and the patient was treated with x-rays Between October 9 and December 19, 1946, he was given a total of 2,000 r (1000 KV) each to 1 anterior and 1 posterior abdominal port, 3000 r. (250 KV) to the left supraclavicular area, and 2,000 r. (1000 KV) to the right, posterior midesophageal region, as well as multiple blood transfusions. During and following this treatment, there was some decrease in the size of the lower esophageal tumor and some symptomatic improvement, which lasted about a month; then the abdominal and back pain recurred and jaundice appeared. He was given 1000 r. to 2 anterior and 2 posterior abdominal ports with no improvement On January 16, 1947, he was admitted to the hospital for HN2 therapy. At that time his hemoglobin was 12 7 Gm, white blood cell count 6,700, and serum bilirubin 7.1 mg. per hundred cubic centimeters. From January 17 to 20, 1947, he received a total of 22 mg of HN2 (0 4 mg./Kg.) Following this treatment, his pain was partially relieved, his appetite improved, and he felt generally better On February 20 the blood bilirubin was 2 9 mg per hundred cubic centimeters

The symptomatic remission lasted about 4 weeks, after which abdominal and back pains again became severe Between February 20 to 23 he received a total of 40 mg of HN2 (0 7 mg /Kg) A maximum leukopenia of 2,700 occurred on March 5. He experienced a good symptomatic remission, and a series of gastrointestinal roentgenograms on February 25 showed interval improvement manifested by less narrowing in the lower esophagus and a generally smoother outline This remission lasted about 5 weeks, but near the end of March the abdominal and back pain and jaundice recurred

During the next 2 months the patient was treated with 2 nitrogen mustard derivatives (of the same order of effectiveness as HN2) and x-rays, but the therapeutic results were brief and unsatisfactory and the jaundice became more severe His course was progressively downhill and he died on July 15, 1947, 17 months after the clinical onset of his disease and 6 months after the beginning of HN2 therapy At autopsy, a gelatinous adenocarcinoma of the cardia of the stomach was found, with extensive metastases to the esophagus and to the thyroid, adrenals, liver, pancreas, pericardium, lungs, and lymph nodes The tumor obstructed the common bile duct and the pancreatic duct

In 4 patients with metastatic carcinoma of the breast there was no substantial response to HN2 (209), but in 1 patient with pleural metastases fewer thoracenteses were required following HN2 (94, 95) No real improvement has been found in a small group of patients with ovarian tumors or with carcinoma of the cervix In 1 patient with an adenocarcinoma of the uterus, metastatic to the liver, abdominal wall, and retroperitoneal nodes, therapy resulted in transient but definite regression of the masses, decrease in pain

these cases, HN2 may often alleviate symptoms and produce objective evidence of improvement for short periods; edema of the diseased area, which may sometimes be a serious complication of x-ray therapy, has not attended the use of HN2 in our experience.

The combination of roentgenotherapy and HN2 has been tried in order to produce an additive injury to lung tumors but the results have been inconclusive (231). This procedure, however, has not had an adequate or critical trial.

HN2 has been given intrapleurally to 2 patients with pleural effusions due to bronchogenic carcinoma (131). In one case, a previously intractable pleural effusion ceased to form, in the other, the HN2 was without local effect It seems likely that if the rapid absorption of HN2 from the pleural space is impaired, it will react locally and an obliterative pleuritis will be produced; if the HN2 is rapidly absorbed the local reaction is unimportant.

Other Types of Neoplastic Disease. A number of patients with other types of cancer than those already discussed have been treated with HN2 (15b,24,94,95,123,131,153a,191,209,224,230,231a). A review of the experiences with these tumors may give some idea of the nature of the therapeutic results.

Metastatic carcinomas of tongue, floor of mouth and larynx do not show an appreciable response to HN2 (131,209), but transient regressions have been induced in carcinomas of the nasopharynx The few cases of pancreatic and colon carcinomas treated with HN2 showed no response Occasionally, there was some transient improvement in swallowing and intestinal symptoms in patients with carcinomas of the esophagus and stomach. The history of one patient with an adenocarcinoma of the esophagus, who demonstrates the most satisfactory response we have seen in gastric carcinoma, is cited in detail (131)

Case 12 R D, a 30 year old male, became aware of a pain in the right lumbar region in February, 1946, which recurred intermittently and became more severe In August, 1946, he noted that the pain was aggravated by eating Anorexia and dysphagia developed, and the patient lost 40 pounds in weight When seen in September, 1946, he appeared to be well nourished but chronically ill A left supraclavicular node was palpable There was some epigastric tenderness but no masses were felt A gastrointestinal examination showed a tumor of the lower esophagus and evidence of mediastinal and extra-esophageal involvement The stools were positive for occult blood Hemoglobin was 6 0 Gm, and white blood cell count 10,400 Esophagoscopy showed an

periods of 2 to 4 months with HN2 (123,209), but there is no evidence of an interruption in the course of the disease. No substantial improvement has been reported from the use of HN2 in primary tumors of bone (209). One patient with a neurogenic sarcoma of the thigh metastatic to bone obtained relief of pain from 2 courses of HN2 (131). Two patients with the multiple hemorrhagic sarcoma of Kaposi, associated with Hodgkin's disease in one case, failed to respond to HN2 (107,171).

Tumors in children, including Wilms's tumor and neuroblastoma, often showed striking regressions from a course of HN2. This improvement was usually very brief, ranging from less than 2 weeks to 2 months (209).

The incidence of beneficial effects from HN2 in these miscellaneous tumors is difficult to ascertain until a large series of cases can be reviewed In one series of 9 patients with miscellaneous types of tumors 4 (44 per cent) were reported to have shown transient subjective improvement (24) In another report (131), 7 out of 17 patients (41 per cent) obtained some symptomatic relief, and in 5 cases (30 per cent) objective evidence of tumor regression was found. The duration of improvement averaged about 1 month, and 3 of the 7 patients responded to a second course of treatment. There was no evidence in these cases that HN2 substantially interrupted or prolonged the course of the disease

No definite indications have been established for the use of HN2 in these diversified tumors. Tumors that are rapidly growing or appear anaplastic histologically may regress rapidly as a result of HN2 therapy. Disabling symptoms, due to the extension of tumors into symptom-producing areas, may be temporarily relieved While symptomatic improvement may occur in these selected cases, the remissions are disappointingly brief, the patient's general condition is usually not appreciably altered, and it often seems as if the treatment has only succeeded in prolonging an unsatisfactory existence. Furthermore, the nausea and vomiting produced by HN2 has, in some instances, appeared to increase the patient's discomfort and to hasten the deterioration in his general condition. It is clear that HN2 should not be tried indiscriminately in all patients with inoperable cancer, or as a last resort in the terminal stages of cancer. Its trial may be justified in carefully selected patients in whom,

and increase in appetite and strength following 2 courses of HN2, each of 0.5 mg. per kilogram of body weight, given 1 month apart (131); 6 weeks after the second course, she died suddenly. At autopsy, massive areas of tumor necrosis and hemorrhage were found in the liver, and these were possibly related to the immediate cause of death.

In 3 of 4 patients with metastatic tumors of the testes, there was a transient reduction in the abdominal and pulmonary metastases, but the general condition of the patients was not significantly improved. The response of a small group of patients with bladder carcinoma and with hypernephroma was poor. Two of 3 patients with metastatic carcinoma of the prostate, no longer responding to estrogens or castration, obtained some temporary relief from HN2 therapy (131). One patient was a 45 year old man with bone metastases who obtained a remission of 2 months following orchiectomy; estrogens were then given, but they proved of little value. He became bedridden with severe sciatic and lumbar pain requiring the frequent use of narcotics Following a course of HN2, totaling 0 5 mg per kilogram of body weight, substantial relief of pain occurred for about 5 weeks, and he was ambulatory during this period. He relapsed, and died 2 months later. The second case was that of a 70 year old man who had been castrated in 1943 for carcinoma of the prostate; 3 years later pain developed in his right hip, and over the right sacroiliac region a swelling appeared, which on roentgenography was found to be an osteolytic lesion of the ilium. An aspiration biopsy showed it to be a metastatic carcinoma from the prostate. He was treated with estrogens and x-ray therapy, without appreciable relief Two courses of HN2 (each of 0 4 mg./Kg) given 3 weeks apart, resulted in considerable relief of pain and a reduction in the swelling in the sacroiliac region The total improvement lasted about 2 months It seems likely that HN2, in damaging the tumor cells by a mechanism different from that of the estrogens, may produce some relief of pain for short periods of time in an occasional patient no longer responding to hormonal therapy.

HN2 was without appreciable value in malignant melanoma, although in 1 patient some symptomatic relief associated with a temporary shrinkage of the neoplasm was reported (209) Some relief of pain has been produced in patients with multiple myeloma for

seemed to have some therapeutic activity, neurologic manifestations appeared at effective doses.

Summary

Nitrogen mustard represents a new and valuable agent for the management of inoperable neoplastic disease. Methyl bis(2-chloroethyl)amine hydrochloride (HN2), the nitrogen mustard in clinical use, is a systemically acting cell poison most closely related in its therapeutic effects to total body irradiation. Its advantages over total body irradiation are that it is less expensive, easier to administer, its toxic and therapeutic effects develop more quickly, tissue recovery is more rapid, and its dosage can be more safely controlled so that maximum therapeutic doses are possible Total body irradiation causes less nausea and vomiting, and its therapeutic effects may be more prolonged

HN2 is an effective, temporary, palliative agent in Hodgkin's disease, lymphosarcoma, chronic leukemia, polycythemia vera, mycosis fungoides, primary lung carcinoma and, to a lesser degree, in other miscellaneous neoplastic disorders. There is little evidence, however, that it alters the course of these diseases or appreciably prolongs life Its great value is as an adjuvant to x-ray therapy in the palliation of neoplastic disease X-ray therapy is more effective in treating localized disease. (When the disease is widely disseminated and unresponsive or unsuitable for extensive x-ray therapy, HN2 may be used in an attempt to control the diffuse or inaccessible disease, while x-ray treatment continues to be available to treat circumscribed areas) HN2 should be given only when definite indications exist. Because of its toxicity and unpleasant side effects, its indiscriminate trial in patients with inoperable cancer or in the terminal stage of the disease is entirely unjustified.

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despite an episode of additional discomfort, it may be expected to produce some useful symptomatic relief, even if only for a brief period

Miscellaneous Disorders Four cases of Boeck's sarcoid have been treated with HN2 (193). The patients received 2 courses, each totalling 0.4 mg per kilogram of body weight, approximately 1 month apart. This treatment appeared to cause an interruption in the course of the disease, and striking improvement was seen in 2 of the patients; but in view of the normal variations in the course of this disease, definite conclusions as to the value of HN2 could not be drawn

Osborne *et al.* (171) treated 1 patient with chronic disseminated lupus erythematosus, which seemed to be chiefly confined to the skin, with a resultant remarkable regression of the skin lesions, a 30 pound gain in weight and a general improvement in well-being which has continued for 9 months thus far. In one of our patients, who had severe psoriasis incidental to carcinoma of the lung, a course of HN2 caused a rapid diminution in the psoriatic lesions. It is likely that the parenteral use of HN2 may be of value in some generalized diseases of the skin Osorio (172) has used HN2 as an effective sclerosing agent in the treatment of varicose veins

Experience with Nitrogen Mustard Derivatives No nitrogen mustard derivative having greater therapeutic activity than HN2 has been described Tris-(2-Chloroethyl)amine hydrochloride has received an extensive clinical trial (74,133,183,227) It is more toxic than HN2, but the relation between toxicity and therapeutic activity is similar to HN2 It has caused venous thrombosis more frequently than HN2 in our experience (133), although Wilkinson and Fletcher (227) are of the opposite opinion Burchenal (45) has reported on two nitrogen mustard derivatives SK 136 (or 1,3-propanediamine-*N*,*N*,*N'*,*N'*-tetrakis(2-chloroethyl)dihydrochloride, and SK 137 (or 1,3-propanediamine-2-chloro-*N*,*N*,*N'*,*N'*-tetrakis(2-chloroethyl) dihydrochloride, in the treatment of leukemia The therapeutic effects appeared to be similar to those which would have been expected from HN2 These agents seemed to cause less nausea and vomiting than HN2, but produced more dizziness, and SK 137 also occasionally caused brief toxic psychoses Boyland *et al* (42) used dimethyl(2-chloroethyl)amine hydrochloride in 3 patients with carcinoma of the lung This agent has a low toxicity but, while it

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Use of Radioactive Isotopes in Medicine

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Introduction and Historical Survey

THE ATOM

Although isotopes, both stable and unstable, have been known for many years, events of the past few years have stimulated great interest in their uses in metabolic investigations, in therapy, and in study of the action of radiation on living tissue.

The isotopes of a given element vary slightly in their nuclear masses and yet behave similarly as far as biologic or chemical reactions of that element are concerned A brief picture of the modern concept of the atom will help clarify this An *atom* consists of a central core or nucleus, about 10^{-13} cm. in diameter, surrounded by a relatively large empty space, about 10^{-8} cm in diameter, outside of which swarms the electron cloud. The nucleus contains practically the entire weight or mass of the atom and is composed of *protons*, units of mass with a unit positive charge, and *neutrons*, units of mass without charge. Each neutron or proton weighs approximately 1840 times the *electron*, the negative unit of charge, which thus has a relatively small mass The number of electrons in the electron cloud is the same as the number of nuclear protons, so that the complete atom is electrically neutral Chemical properties of an atom are functions of the outermost orbital electrons, disturbances in the outer group also give rise to the optical spectra, and changes in the inner group of electrons near the nucleus give rise to the characteristic x-rays of the element The neutrons in the nucleus lend mass but no charge to the atom, and hence variation in the number of neutrons in the nucleus usually does not affect the chemistry of the atom since the electron cloud remains unaltered. (A few of the lightest elements, especially hydrogen, are exceptions to this statement.) An *isotope* is a nuclear species with a fixed charge and mass. The number of protons in the nucleus is called the *atomic number*. Thus, an element with two or more isotopes consists of atoms with

cause of the relative ease and inexpensiveness associated with the assay, will probably supplant the stable isotopes of these elements in tracer work. In the case of hydrogen, it is still not clear what relative importance the stable isotope, deuterium, and the radioisotope, tritium, will have in tracer work. Of the 274 stable isotopes only 10 have been applied to problems in the living organisms, these being isotopes of the 9 elements, $_1H$, $_2He$, $_3Li$, $_4Be$, $_5B$, $_6C$, $_7N$, $_8O$, and $_{16}S$. Isotopes of $_2He$, $_3Li$, and $_5B$ have not been used as tracers, but, because of their peculiar properties, as adjuvants of radiation experiments The lithium and boron nuclei, Li^6 and B^{10}, undergo a process of splitting when bombarded with fast particles, and the ionization produced by the products is greater than that produced by the incident particles Localization of compounds containing lithium and boron in and around tumor tissue has made possible selective radiation of the neoplastic tissue (244) Bombardment of beryllium with protons or deuterons causes the emission of fast neutrons, and this beam has been used in the therapy of cancer (214,215). In the case of helium, the use of beams of alpha particles, or nuclei of He^4, has been used in testing the biologic effects of accelerated nuclear particles (245). Similar use has been made of, or suggested for, protons (239) and deuterons (220), the nuclei of the two isotopes of hydrogen.

The use of the stable isotopes of the light elements, $_1H$, $_6C$, $_7N$, $_8O$, and $_{16}S$, for which adequate separation methods have been devised, has elucidated many problems in biology and medicine. Schoenheimer (194), Rittenberg (191), Hevesy (112,113), and many others have demonstrated the dynamic state of body constituents, and many problems in intermediary metabolism have been solved through use of stable isotopes. But there have been certain limitations or relative disadvantages in using this type of tracer· (*1*) difficulties in the methods of separation and measurement (mass spectroscopes are delicate and expensive instruments), (*2*) the ready availability of radioactive isotopes for most of the elements, (*3*) the greater sensitivity of the tracer method when radioisotopes are employed Until recently the useful stable isotopes have been restricted to the light elements; now, however, the U.S Atomic Energy Commission (4) has announced the availability of a large series of separated isotopic species for most of the elements of biologic

the same atomic number, but two or more nuclear masses. Between 700 and 800 different isotopes of the 96 elements are now known, all elements having three or more known isotopes

STABLE ISOTOPES

Isotopes may be stable or unstable. Although the existence of more than one stable form of an individual element was definitely predicted as early as 1910 by Soddy, proof was lacking for the lighter elements until the work of Aston in 1920. Subsequent successful separation of the stable forms of certain elements permitted their use as "tracers." The use of a stable isotope in labeling a substance and tracing its metabolic course through the body depends on the fact that naturally occurring elements have a uniform isotopic composition. Any variation in the concentration of the isotopes, usually an increase in percentage of a less abundant form, will "label" the element or compound, which can then be followed in its wandering by suitable instruments Thus, naturally occurring sulfur contains four stable isotopes as follows.*

Isotope	S^{32}	S^{33}	S^{34}	S^{36}
Abundance	95.1%	0.74%	4.2%	0.016%

Each of the above atoms contains 16 nuclear protons, and hence all belong to the same element sulfur, yet variation in the number of nuclear neutrons has created four different stable isotopes If one were to use stable sulfur as a tracer in a chemical or biologic experiment, it might be done by increasing the concentration of any of the rarer isotopes, S^{33}, S^{34}, or S^{36} Already, S^{34} has been so used (41).

Much important and fundamental work in biology has been done using the stable isotopes of hydrogen, carbon, and nitrogen; and a little work has also been carried out with stable forms of oxygen and sulfur It seems likely that further tracer work with isotopic nitrogen and oxygen will be confined to the stable isotopes, but satisfactory radioisotopes of carbon and sulfur are now available and, be-

* We shall designate the *atomic number* by appending it as a left subscript to the chemical symbol e g , ${}_{15}P$, ${}_{1}H$, ${}_{92}U$ Similarly, the *mass number* (the number of neutrons and protons in the nucleus) is appended as a right superscript to the chemical symbol when a particular isotope is specified, e g P^{31} or ${}_{15}P^{31}$, ${}_{1}H^{2}$, etc

1923, Hevesy (111) reported on the use of a radioisotope of lead as an indicator of lead metabolism in plants. By substitution of the radioactive isotope for the stable form he was able to measure the uptake and distribution of the element in various parts of the plant. The precision of the measurements enabled him to use only minute quantities of the metal, and thus the toxicologic effects of lead on living tissue were avoided.

Prior to these investigations the only means available for attacking a metabolic problem involving a stable element was by a direct chemical approach Study of the absorption, transportation, and utilization of various elements and compounds necessitated administration of these substances in amounts large enough to be detected in the tissues or end products by suitable macrochemical or microchemical methods Since the normal constituents of a living organism vary only slightly, the chemical and physiologic systems were usually disturbed by this approach, and the results obtained were by no means a true picture of the metabolism of these substances In addition, studies of this type could not discriminate be-

sue metabolite

Artificial Radioactivity

The discovery of artificial radioactivity by Joliot and Curie in 1933 and the development of the cyclotron by Lawrence, whereby artificial radioactive isotopes of most of the elements could be produced with relative ease, initiated a new phase in biologic investigations

The transmutation of elements in the cyclotron is usually effected by bombarding atoms of one element with positively charged particles that have been accelerated to energies sufficiently high to penetrate the nuclear barrier Neutron beams secondarily produced by proton or deuteron bombardment of beryllium may also be used When an accelerated particle enters a nucleus, it forms an unstable

importance, and considerable extension in the use of stable isotopes may be anticipated. For discussion of stable isotopes as applied to biology and medicine, the reader is referred to the new standard books on tracer methodology by Kamen (123), Siri *et al.* (206), and Hevesy (112), and the reviews by Dougherty and Lawrence (39a–b).

RADIOACTIVE ISOTOPES

Natural Radioactivity

The discovery of radioactivity by Becquerel in 1896 began a new branch of science that many years later culminated in the development of the atomic bomb and the chain-reacting pile. A few years after Becquerel's observations on the peculiar penetrating rays associated with uranium, the Curies identified radium and polonium; and within the next decade, other sources of radioactivity, including actinium, thorium, radiolead, potassium, and rubidium were discovered To explain this phenomenon of radioactivity, Rutherford and Soddy formulated the theory of atomic transformation which has become one of the basic concepts of physics

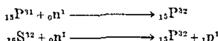
The use in medicine of these new radioactive elements was not long in following their discoveries Thus Walkhoff (234) and Giesel (73), in 1900, reported on the physiologic effects of the radiations from radium and its decay products on tissues, and within a few years these radiations had been found to be effective in the treatment of neoplastic disease and certain dermatoses

Elucidation of the nature of the rays given off by these radioactive elements occurred within the next 20 years. The genetic relationship of the natural radioactive elements through three different decay schemes was established The uranium, actinouranium, and thorium families of radioactive elements, after a series of emissions of alpha and beta particles and gamma rays, end in a particular isotope of lead—of atomic weights 206, 207, and 208, respectively. (The relative abundance of different isotopes of lead in ores has been utilized in estimating the age of the earth)

The recognition by Hevesy and Paneth (117) of the possibilities possessed by radioactive isotopes as indicators of biologic processes introduced a new use which was to become by far their most important application—namely, as tools in tracer methodology. In

tribution of adequate amounts of many of the isotopes at a very low cost

For example, P^{32} may be produced in the pile much more cheaply than in the cyclotron by either of the following reactions·



$$ {}_{15}P^{31} + {}_{0}n^{1} \longrightarrow {}_{15}P^{32} $$

$$ {}_{16}S^{32} + {}_{0}n^{1} \longrightarrow {}_{15}P^{32} + {}_{1}p^{1} $$

The second of these reactions has the great advantage of producing *the unstable species* P^{32} *essentially uncontaminated with the stable* species P^{31}. It is said to be *carrier-free.*

Since most unstable isotopes emit beta particles (high speed electrons), or gamma rays (electromagnetic energy), or both, which can be readily detected with suitable measuring devices, an exquisitely sensitive method of following the fate of an element is thus available. The Geiger-Muller counter is the detecting device most usually employed.

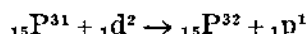
During the last few years the bibliography of isotopes in medicine *has become so extensive that complete coverage of the field is almost* impossible. An attempt will be made here to treat the most important medical applications of radioactive isotopes used as investigative tools and in therapy. The technic of tracer methodology can be referred to only briefly, but the advantages and potentialities of these methods will be manifest from a consideration of what accomplishments their use has already permitted.

General Aspects of the Use of Radioactive Isotopes in Medicine

The chemical properties of a radioisotope are virtually indistinguishable from those of other isotopes of the same element The radioisotopes and stable isotopes of each element except the lightest behave similarly in all metabolic and physiological processes, provided that, in the case of radioisotopes, the radiation emitted has been kept below that level at which detectable changes induced by radiation may occur Since the detection of radiations is much more sensitive than the finest microanalytical procedures, doses of a labeled substance can be given that are in most cases well within the normal physiological limits of the body.

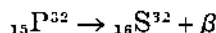
or compound nucleus that decays almost immediately by the emission of one or more heavy particles—such as protons, neutrons, or alpha particles With the earlier cyclotrons these changes were relatively simple but with the new supercyclotrons the compound nucleus may split off complex fragments composed of proton–neutron groups The resulting nucleus may be stable or unstable and, if the latter, undergoes decay with a characteristic *half-life* The ejected particles from these radioactive nuclei may be photons* alone, positive or negative electrons alone, or combinations of photons and electrons. In certain cases the instability of the nucleus is adjusted not by the ejection of a particle but by the capture of an electron from the innermost or K electron shell (by so-called "K-capture"), which is always accompanied by the emission of characteristic x-rays The half-life of these decay processes is the time necessary for half of the atoms of an unstable isotope to decay, and is rigidly fixed for any given isotope. Radioactive half-lives range from a small fraction of a second to over a hundred billion years.

The first use of artificial radioactive isotopes in biology was reported by Chiewitz and Hevesy (23) in 1935 They studied the metabolism of phosphorus in rats by means of the radioactive isotope P^{32}, *and demonstrated its deposition in bones.* Radioactive phosphorus may be formed in the cyclotron by the bombardment of stable phosphorus (P^{31}), with deuterons, as follows:



$$_{15}P^{31} + {}_1d^2 \rightarrow {}_{15}P^{32} + {}_1p^1$$

A neutron is thus gained by the stable phosphorus atom with the formation of the radioactive isotope P^{32} Radiophosphorus emits a negative electron or beta particle, has a half-life of 14 3 days, and ultimately becomes transformed into an atom of sulfur, as follows



$$_{15}P^{32} \rightarrow {}_{16}S^{32} + \beta$$

Although immediate use of the artificial isotopes as tools in biologic investigation followed, the quantitative limitation in supply and the expense involved in their production necessarily curtailed widespread application of these powerful new agents. These limitations were to a great extent overcome by the development of the chain-reacting pile (1942) and the subsequent production and dis-

* "Photon" is the name applied to a *unit* of gamma radiation, which is electromagnetic radiation of the same nature as x rays and light

quately shielded with lead, fairly accurate localization of the isotope may be expected Sodium, potassium, short-lived carbon, iodine, bromine, chlorine, and the noble gases have been used in various experiments employing this technic. The hard beta ray emitters, such as phosphorus and strontium, can similarly be detected with the *in vivo* detection devices Radioactive phosphorus emits a beta ray and decays to sulfur The beta particle has a maximum energy of 1 7 million electron volts (m e v.), and a maximum range in tissue of about 8 mm. with a mean path in tissue of about 1 mm. Thus, if the deposit of radioactive phosphorus is situated close to the surface of the body, its radiations can be detected by a sensitive G-M counter tube Although a discussion of detection devices is beyond the scope of this chapter, it is apparent that the counter tube employed will vary, depending upon whether gamma or beta rays are emitted; the latter require a tube made of much thinner material, such as glass or mica, and must be applied much closer to the tissue exam- [illegible] malignant neoplasms (147,149).

[illegible] ng the isotopes emitting the gamma and hard beta rays are well suited for the *in vivo* detection technic, with it serial studies on the same structure can be made, and the procedure is simpler than the other technics available. In addition, with constant progress in instrumentation it should become possible to survey deeply situated tissues or lesions with carefully built detecting tubes If localization in tumor tissue of a sufficiently high degree is ever achieved for an isotope, the G-M tube developed by Strajman (216) should prove extremely useful. These tubes can be made small enough to fit into proctoscopes, sigmoidoscopes, bronchoscopes, and the like, and thereby small lesions impossible to detect externally can be surveyed by intimate approximation of the counter tube. Preliminary studies with radiophosphorus on cancerous lesions have already shown the applicability of this diagnostic approach (147,149).

In Vitro Technic

The second method used to assay radioactive materials is the *in vitro* technic With this method specimens removed from the patient are assayed for radioactivity after preparation suitable for the par-

DETECTION

In Vivo Technics

Incorporation of a radioisotope in a molecule permits the tracing of a substance after its administration by one of three technics, depending on the radiation emitted by the isotope and the type of analysis required. Some of the earliest tracer studies in human beings employed the *in vivo* radioactive tracer method (97). In this method the patient is submitted to direct measurement and the labeling isotope is followed by applying a Geiger-Muller tube to those regions of the body suspected of accumulating the radioelement—as, for example, the thyroid in the case of radioiodine. Many tissues or organs may thus be studied, and reliable quantitative data may be obtained if the geometric factors of the detection procedure are known with sufficient accuracy. The appearance of increased activity in the extremities as detected by counter tubes grasped in the hands or placed against the feet following oral administration of the radiohalogens early served as an index of the absorption of these elements from the gastrointestinal tract (96). In peripheral vascular diseases, interesting work with radiosodium has utilized the *in vivo* detection technic to determine variations in the circulatory pattern of the extremities (183,184), while other studies with sodium have demonstrated changes in the arm-to-leg circulation time in various pathologic states (207). Much work on the uptake and distribution of radioiodine has been performed, employing this method of analysis In addition to uptake curves in normal and abnormal states as determined by external counters placed in positions geometrically favorable for recording the activity of the radioiodine, modifications of the method have resulted in "profile studies" that help to evaluate the causes of abnormal iodine distribution (59). The above experiments will be more fully discussed in the later sections.

The *in vivo* detection technic is dependent upon the type of radiation emitted by the isotope Those isotopes that emit gamma rays, the penetrating electromagnetic radiations akin to x-rays, can be detected, after administration, by suitable instruments placed on or close to the skin In practice, the instrument most frequently used is a Geiger-Muller tube connected to a suitable electronic circuit for amplification and recording If the G-M counter is ade-

TABLE I (*continued*)

Element	Isotope	Problems to which isotope has been applied
Strontium	Sr^{85}, Sr^{89}, Sr^{90}	Absorption, distribution, and excretion; bone physiology; therapy of bone sarcoma
Yttrium	Y^{86}, Y^{91}	Absorption, distribution, and excretion; colloids in reticulo endothelial system
Zirconium	Zr^{89}, Zr^{95}	(as above)
Columbium	Cb^{95}	(as above)
Antimony	Sb^{124}	Absorption, distribution, and excretion; experimental filariasis
Iodine	I^{130}, I^{131}	Iodine metabolism, thyroid physiology; treatment of hyperthyroidism and thyroid carcinoma; diagnosis of brain tumor (di
Gold	Au^{198}	
Astatine	At^{211}	Thyroid physiology
	B. NATURAL RADIOISOTOPES	
Lead	Pb^{210}(RaD), Pb^{212}(ThB)	Absorption, distribution, and excretion
Bismuth	Bi^{210}(RaE), Bi^{214}(RaC)	Absorption, distribution, and excretion; velocity of blood flow
Polonium	Po^{210}(RaF)	Distribution and excretion, effect in reticulo-endothelial system
Radon	Rn^{220}(Tn), Rn^{222}†	Absorption, distribution, and elimination (in breath), treatment of various malignancies and skin disorders
Radium	Ra^{224}(ThX), Ra^{226}†, Ra^{228}($MsTh_1$)†	Absorption, distribution, and excretion, treatment of various malignancies and skin disorders, toxicity studies
Thorium	Th^{228}(RdTh), Th^{230}(Io), Th*, Th^{234}(UX_1)	Absorption, distribution, and excretion, toxicity
Uranium	Natural element, U^{235}	Production of slow neutrons; colloids localizing in reticulo-endothelial system, effects of *in vivo* fission

In addition, isotopes of some 16 elements have been used for preliminary tracer studies on their fate in the vertebrate body—namely, Be^{7}, Se^{75}, Mo^{99}, Ru^{103}, Ru^{106}, Te^{127}, Te^{129}, Cs^{134}, Ba^{133}, Ba^{140}, La^{140}, Ce^{141}, Ce^{144}, Pr^{143}, Pm^{147}, Pa^{233}, Np^{239}, Pu^{239}, Am^{241}, and Cm^{242} Also, Mg^{x} has been used to study photosynthesis

* Elements essential in human nutrition

† Radiation effects partially, or entirely, due to decay products

avoid considerable errors due to self-absorption of the radiations by the thickness of the material. Extremely thin samples of radio-iron, -gold, -cobalt, -zinc, etc. have been prepared by electroplating the

TABLE I

Artificial and Natural Radioactive Isotopes Used in Medicine

A. ARTIFICIAL RADIOISOTOPES

Element	Isotope	Problems to which isotope has been applied
Hydrogen*	H^{3}	Body water content; photosynthesis
Carbon*	C^{11}	CO_2 metabolism in plants and animals; CO distribution and elimination
	C^{14}	Photosynthesis; tracer application of synthesized organic compounds
Nitrogen*	N^{13}	Respiratory gas exchange
Fluorine*	F^{18}	[illegible]
Sodium*	Na^{22}	[illegible]
	Na^{24}	[illegible]
Phosphorus*	P^{32}	[illegible] lism; therapy of chronic leukemia, polycythemia vera, and skin lesions, diagnosis of [illegible] the ret-
Sulphur*	S^{35}	[illegible] metabo-
Chlorine*	Cl^{36}	Mineral metabolism
Argon	A^{41}	Respiratory gas exchange; blood circulatory pattern
Potassium*	K^{42}	[illegible]
Calcium*	Ca^{45}	[illegible]
Manganese*	Mn^{52}	[illegible] en
	Mn^{54}	
	Mn^{56}	
Iron*	Fe^{55}	Mineral metabolism, blood physiology; blood preservation
	Fe^{59}	
Cobalt*	Co^{56}	Mineral metabolism, radioactive cobalt wire in therapy
	Co^{57}	
	Co^{58}	
	Co^{60}	
Copper*	Cu^{64}	Mineral metabolism
Zinc*	Zn^{63}	Mineral metabolism in normal and abnormal states, local irradiation of neoplasms
	Zn^{65}	
Arsenic	As^{74}	[illegible] ex
Bromine*	Br^{82}	[illegible] gy;
Krypton	Kr^{79}	Respiratory gas exchange, blood circulatory pattern
	Kr^{81}	
Rubidium	Rb^{86}	Electrolyte exchange

Table continued

ticular isotope being studied With the soft beta ray emitters such as long-lived carbon, sulfur, and iron isotopes, extremely thin samples of material must be prepared or corrections made in order to

Since the limit of resolution with this method is about 25 microns, recent technics have been developed to permit closer approximation of tissue and emulsion Bélanger and Leblond (9) added melted photographic emulsion upon a tissue section; Evans (54) mounted the histologic section directly upon a photographic plate, whereby exposure was permitted, and the tissue was then stained Stripped emulsions (177) have also been used with good results, improving the resolving power to 5–10 microns.

For excellent reviews of radioautography, the reader is referred to the articles by Gross and Leblond (81) and Axelrod (5).

SURVEY OF RADIOISOTOPES USED IN MEDICINE

The more important radioactive isotopes used in medicine or related fields, and their applications, are listed in Table I, condensed and amended from Dougherty and Lawrence (39) and brought up to date. This table contains isotopes of some 35 elements; in addition, those of 17 more are listed at the end of the table, the latter being for the most part those for which only preliminary tracer studies have been carried out. Thus, radioactive isotopes of some 52 of the 96 elements have so far found use in biology and medicine. Although many elements have not yet been studied with radioisotopes, convenient species of a number of additional elements are available in quantity from the pile, and it will doubtless not be long before a number of these have been used in experimental medicine

Certain important isotopes can be produced only in the cyclotron, whereas others can also be produced in the pile The cyclotron is far more versatile in the types of reactions that it can effect—hence in the number of different products However, for many radioactive species that can be produced by the pile, the latter instrument is far more efficient, producing much larger quantities of the isotopes in question Table II* lists the most important isotopes, their main characteristics, and whether or not they are available from the pile Most of those produced in the pile are now available to qualified laboratories through the U S Atomic Energy Commission (3).

Radioisotopes have two main categories of application in medicine—as tracers of metabolic and other physiologic processes (in common with stable isotopes), and as agents for administering vari-

* Pages 142–145.

isotope from a solution onto copper, aluminum, or platinum cathodes (40,237). Measurements made in the case of iron, with the addition of varying amounts of carriers to the electroplating solution, have shown that 5–10 mg. of inactive iron can be added without changing the number of counts detected.

P^{32} and Sr^{89} emit hard beta rays and more leeway in thickness of the sample is permitted. Those isotopes emitting gamma rays can be measured with little attention paid to thickness of the specimen, thus quantitative determinations in radioiodine excretion studies can be easily made without any special chemical procedures by counting the gamma rays in a large sample of urine. Marinelli (154) has devised a technic in which the beaker of urine completely surrounds the G-M tube, giving greater efficiency in the counting rate of the specimen. More preparation of a sample is usually required for quantitative measurements of the beta particles than for the assay of the gamma rays

The *in vitro* method probably comprises the most useful technics for obtaining the precise quantitative data necessary for fundamental investigations

Radioautographic Technics

The third method utilized in experiments to localize radioactive isotopes is the radioautographic technic The ionizing radiations emitted by most radioactive isotopes can be used to render the grains of a photographic emulsion developable, and fairly precise distribution patterns of certain isotopes in a tissue may be obtained Although the data are more or less qualitative, it has been of great value in medical investigation The tissue is placed in contact with a photographic film protected from fogging by some suitable light-tight box or paper, and, after the lapse of the necessary time interval, the film is developed Blackening of the film occurs at those points where the radioisotope has accumulated in the tissue The method of approximating whole blocks of tissue or tissue sections to "no screen" x-ray film or dental film has proved fairly adequate for some problems in the hands of many workers The presence of a greater amount of radium in bones than in soft tissues was early shown by this method (66), and numerous other studies on bone and tissue deposition and metabolism have been carried out by means of radioautographs (31,102,133,175,176,223).

been made that we feel will prove useful in the overall orientation of the reader. Tracer applications that have been suggested as diagnostic procedures are deliberately excluded from consideration here, but are taken up in the next section

In Table I the material of this section is organized in outline according to element. Here it seems more useful to consider work with isotopes according to major physiologic categories.

INTERMEDIARY METABOLISM

Carbohydrates and Fats

The labeling of carbohydrates and fats with radioisotopes has been but little employed. The species H^3 (T), C^{11}, and C^{14} are available, but none has as yet been extensively used insofar as published research is concerned However, now that C^{14}, the long-lived isotope (half-life of about 5,100 years) is available, much research may be expected, indeed, by the time this article appears in print many new papers describing research on carbohydrate and fat metabolism with C^{14} will have appeared

C^{11}, despite its short half-life, has been used to some extent in the study of the assimilation of CO_2 by heterotrophic organisms. In fact, its use by Ruben and Kamen (193) in 1940 made it possible to establish the revolutionary fact that CO_2 in animals is not merely an end product of the metabolism of fats and carbohydrates but is in reality an essential or important substance in certain organic reactions In vertebrates, for example, CO_2 is incorporated into the tricarboxylic acid cycle, and thus in part finds its way into liver glycogen

Although not yet available in quantity, tritium may be expected to prove of considerable ultimate importance, especially when used to label relatively stable hydrogen positions in organic molecules It is conceivable, but not probable, that O^{15} may also have some future application, however, because of its extremely short half-life (126 seconds), its use will always be restricted.

In contrast to the limited application of radioisotopes as direct labels in the study of carbohydrate and fat metabolism, the stable isotopes H^2 (D) and C^{13} have been extensively used O^{18}, and possibly O^{17}, although not used as yet, may also be expected to prove important

ous types of radiation to organisms, tissues, and cells For the most part, the tracer application is best suited to basic experimentation, although a number of promising diagnostic procedures have recently been reported, using tracer technics So far, radioisotopes of 51 elements have been used as tracers in studying mammalian physiology. On the other hand, as agents of irradiation, isotopes have primary importance in the clinical field—that is, in therapeutics, although it must be noted that mature clinical application can come only as the result of much basic experimentation in the general field of the biologic effects of radiation and on the specific properties of individual isotopes themselves. A third general application of isotopes may also be mentioned—namely, their use as quantitative tools, biochemical analysis by the *isotope dilution technic* is now a well-established adjunct in experiment biology (112,123).

The use of isotopes as tracers has almost endless possibilities Their principal advantage, of course, is the fact that biochemical processes can be studied in the normally functioning organism, and processes of intermediary organic metabolism and of electrolyte dynamics can be followed which are otherwise quite beyond the reach of other technics of biologic investigation In theory, it is possible to label any metabolic process—from strictly normal mechanisms through all stages of abnormal metabolism, both where the labeled substance is itself the agent responsible for the abnormality and where the labeled substance is a normal or abnormal metabolite under the influence of some other deranging agent It is also possible to label physiologic processes that, strictly speaking, are not metabolic—such as blood flow (with inert colloids), the life of red cells (with radioiron), the uptake and distribution of inert gases, the fate of foreign cells, tissues, and even organisms, etc

Radioisotopes as Tracers

Hevesy (112) has recently published a text comprehensively covering the field of tracer application with radioisotopes in animal biochemistry, physiology, and pathology The literature up to and including a part of 1947 has been thoroughly reviewed Such a review is beyond the scope of this paper We can do little more than select the most significant representative studies in the principal fields of animal physiology—studies that illustrate the value of the radioisotopes Certain generalizations of concept and data have

pholipid metabolism. For example, the main site for the elaboration of phospholipids to be used systemically is the liver, but the kidney can synthesize its normal level of phospholipid independently of the liver, and the intestine, brain and muscle are also capable of independent synthesis; furthermore, the liver manufactures all the plasma phospholipids and is the organ mainly concerned with their utilization.

The use of P^{32} in the study of the phospholipids provides one of the best examples of the great values of isotopes as tools in elucidating mechanisms in intermediary metabolism

Phospholipid metabolism can of course be studied with other labeling substances. So far, however, aside from P^{32} only stable isotopes have been so used and only to a limited extent—e g, the work of Boxer and Stetten (15) with N^{15} labeled choline

Proteins

As with carbohydrates and fats (other than the phospholipids), the study of the metabolism of proteins has been largely restricted to the stable isotopes Recent work (65,79,240) has demonstrated the important role that C^{14} is soon to assume. So far, it is the rarer stable isotope of nitrogen, N^{15}, that has been extensively used in studying the metabolism of amino acids and other nitrogenous substances involved in the synthesis and degradation of protein; the stable isotope, C^{13}, has also been used (82,228).

C^{11} has not lent itself to the study of protein metabolism, but C^{14} is ideally suited Already Greenberg and Winnick (79) have studied the metabolism of C^{14} labeled glycine and Winnick *et al* (240) of tyrosine in the rat, they have shown the rapid incorporation of the carbon label into protein after intravenous administration, but the relatively slight exchange with other amino acids, particularly in the case of tyrosine This is not surprising in view of earlier evidence that, in general, it is only the carbon chain or ring in the essential amino acids that is indispensable, rather than the intact, aminated molecule (an exception to this being lysine) Greenberg and Winnick found that at 6 hours glycine was concentrated in the protein of various organs in the following order intestinal mucosa, bone marrow, liver, kidney, plasma, spleen Winnick *et al.* found for tyrosine. intestinal mucosa, kidney, plasma, liver, spleen.

The metabolism of the sulfur-containing amino acids (methionine,

Several excellent reviews have appeared on the use of isotopes in the study of carbohydrate and fat metabolism—by Vennesland (228), and Gurin (82), Buchanan and Hastings (18), Wood, (242) Work, largely with stable isotopes on the metabolism not only of carbohydrates and fats, but of all organic substances in the body, has established the important fact that many simple organic substances of exogenous source must not be regarded as the specific precursors of other, more complex substances, but rather as contributors to the general metabolic pool from which the latter substances are formed This is one of the primary tenets of the concept of a dynamic equilibrium state for organic substances in the body, in which considerable breakdown and resynthesis is continuously taking place without an appreciable net change in the composition of the systems

Recently Putman *et al.* (182) have reported on methods for isolating starch and hexose sugars containing C^{14} from plants exposed to an atmosphere containing labeled CO^2. This has considerable significance inasmuch as the biosynthesis, as opposed to chemosynthesis, of carbohydrates will unquestionably be widely employed to obtain these substances in a labeled form for use in tracer studies

It has for some time been known that the assimilation and utilization of carbohydrates and ordinary fats involves the formation of phosphate esters The radioisotope P^{32} has found some application in the study of this role of phosphate (22,38,112,123) Moreover, in one field of metabolic research—with a class of fatty substances, the phospholipids—the use of P^{32} has been very great indeed.

Phospholipids

The phospholipids, an important class of fatty substances, of which the exact significance, despite considerable study, is still somewhat obscure, all include a phosphate group, which binds an organic nitrogen base, such as ethanolamine and choline, to a substituted complex alcohol This phosphate group is not exchangeable with inorganic phosphate except with the breakdown and resynthesis of the phospholipid molecule Chaikoff and Zilversmit (20) and Hevesy (112) have recently reviewed in detail the use of P^{32} in the study of phospholipid synthesis.

Chaikoff and his group and other workers, using the P^{32} label, have shown many important, largely unsuspected things about phos-

inorganic phosphate is administered to the vertebrate, it is shortly to be found in the nucleic acids of all tissues (105,116,226,227) but the turnover rates are rather different Desoxyribosenucleic acid, which is predominantly a constituent of nuclear nucleoprotein, undergoes a more rapid process of breakdown and resynthesis in such fast-growing tissues as the spleen, regenerating liver, and neoplasm than in slow-growing tissues, such as the adult liver. By contrast, ribosenucleic acid, which is a constituent of cytoplasmic nucleoprotein, turns over rapidly in tissues that are metabolically active, even though cell multiplication may be negligible. A good example of this last situation is the adult liver

The intimate relation between cell division and desoxyribosenucleic acid is strikingly suggested by studies of Ahlström *et al.* (1), who found that in experiments with 3 5 day old rats 2 per cent of the desoxyribosenucleic acid formed in a 2 hour period came from labeled phosphate, whereas just under a 1 per cent increase had occurred in the total weight of this nucleic acid in the organ as a whole This result may be readily interpreted as a consequence of the following mechanism—that every time a desoxyribosenucleoprotein unit is duplicated its nucleic acid moiety breaks down and two are formed in place thereof. However, there is no direct evidence as yet that the turnover of the phosphate group directly parallels the turnover of other constituents of the nucleic acid molecule.

Perhaps no other phase of biochemical study strikes so closely at the central mysteries of the life process as does that of nucleoprotein and nucleic acid metabolism Isotopic tracers are ideally suited to such study, and it can confidently be expected that the near future will see a great expansion in work on this subject.

Vitamins and Hormones

Studies with radioisotopes on vitamin metabolism have been very limited, nor has there been much work with stable isotopes, neither C^{14} nor H^3 has been as yet reported upon as a vitamin label, although their potential importance is obvious However, Borsook *et al* (14) have studied the metabolism of thiamine labeled with S^{35} and have shown that its interchange and destruction in the body are very rapid, just as with the main metabolites—proteins, fats,

cystine, etc.) has been fairly extensively studied with the use of the radioisotope S^{35}. The first of such studies was that of Tarver and Schmidt (217), who used S^{35} to prove that biologic conversion of methionine to cystine can take place. A series of studies followed, particularly by Tarver and his associates, on a variety of problems in sulfur metabolism; the most recent of these studies is that of Friedberg *et al.* (64) on the distribution pattern of labeled sulfur in the proteins and free amino acid fraction of the tissues of fasted rats after intravenous administration of labeled methionine. The sulfur was incorporated in various organs in the following order: intestinal mucosa (not intestinal muscle), pancreas, spleen, kidney, plasma, liver, testis. Only the value for the protein of the erythrocytes rose continuously during the course of the experiment; other organs and tissues showed a downward trend after initial stabilization of the S^{35}.

Studies with C^{14} and S^{35} labeled amino acids have thus revealed that upon either oral or parenteral administration the intestinal mucosa concentrates them to the greatest degree of any tissue, with high values also for the kidney and liver and usually for the plasma and spleen as well. These results were in some cases verification of earlier experiments carried out with N^{15}, H^2, or C^{13}. Such studies on protein formation are the pioneer steps in what will certainly become a vast field of research.

Nucleic Acids

A special class of proteins, the nucleoproteins, has lent itself to study by means of P^{32}, for like the phospholipids, the nucleic acid part of these substances contains phosphate groups which serve to link up organic moieties. The nucleic acids are themselves highly polymerized, complex molecules and have particular significance because in combination with their proteins they are believed to form the basis of self-duplicating units, or genes, that underlie the continuity of the life process itself, both ontogenetically and phylogenetically.

Hevesy (112,113) has recently reviewed the field of nucleic acid metabolism, particularly in reference to its study with isotopes, of which P^{32} has played the major role. The turnover of nucleic acids in various tissues has been rather widely investigated. When P^{32} as

MINERAL METABOLISM

Water

Mineral metabolism is a wide concept, in many cases rather artificially separated at the present time from organic metabolism. It concerns the uptake, utilization, and excretion of water, the function of the electrolytes in body fluids, and the role of the mineral constituents of bone and of the so-called "trace elements"

Studies on water metabolism with isotopes have been almost entirely restricted to deuterium. Both deuterium and tritium suffer somewhat as labels of water because of the relatively great mass differences between them and protium and the consequently significant deviations in the behavior of "heavy" water molecules containing either deuterium or tritium for that of ordinary water molecules. In all likelihood, O^{17} and O^{18} will prove much more useful as labels for the water molecule, especially in intermediary metabolism.

One study of medical importance, however, has appeared with tritium as a label Pace *et al.* (174), have used tritiated water to measure total body water in man In this case the importance of a differential metabolic fate of normal and isotopic hydrogen was negligible. The method employed by Pace *et al* was merely that of the classic isotope dilution technic It seems not unlikely that the oxide of either deuterium or tritium, or both, will come into use for the clinical determination of total body water

Electrolyte Dynamics

The dynamics of electrolyte uptake and distribution can be studied by the usual chemical means only to a limited degree It is, of course, possible to measure net shifts and the rates of body intake and output with general chemical procedures, but, when there is a net over-all equilibrium in the body, the movement of electrolytes between fluid phases, i e," electrolyte exchange, can be studied only with isotopes '

Studies on electrolyte shifts have been made with radioactive ions of sodium, potassium, rubidium, strontium, chlorine, bromine, and iodine, as well as with labeled phosphate (77,85,95) Dougherty and Lawrence (39a–b) have made a cursory summary of this work Hevesy (112) has reviewed it in detail In general, it has been

and carbohydrates. The study of hormones labeled with radioisotopes is a much more developed field if one includes the numerous experiments on thyroid physiology with radioiodine, which, when administered as iodide, is incorporated ultimately into thyroxine, now generally regarded as the essential thyroid hormone. The use of radioiodine is discussed in the subsection on trace elements under mineral metabolism.

In addition to the numerous studies on the thyroid hormone, there are a few on labeled active principles of other endocrine glands.

Recently, Gurin and Delluva (83) have fed rats phenylalanine labeled either with C^{14} in the alpha or carboxyl carbons or with tritium. They recovered labeled adrenalin in both cases and were thus able to give the first direct proof that this hormone of the adrenal medulla can be formed from phenylalanine. The evidence suggested that in the biosynthesis of adrenalin, phenylalanine is decarboxylated and that the resulting amino ethyl chain remains attached to the benzene nucleus.

Radioactively labeled steroids have recently become available, Turner having synthesized cholestenone (225), and testosterone (224), the male sex hormone, both with C^{14} in ring A.

The use of labeled steroids will doubtless provide vital information on the function of the gonads and adrenal cortex in growth, maturation, and general metabolism, and possibly also on neoplasia, in which steroid metabolism appears, in some cases at least, to be deranged

Insulin has been labeled by coupling it with *p*-azoiodobenzene containing radioiodine (188) and the absorption rate of its various forms studied This was found to be, for the preparations used, as follows insulin > globin insulin (with zinc) > protamine zinc insulin Although radiozinc, Zn^{65}, has also been used to label insulin for chemical studies (25a), this radioactive material has not been applied to *in vivo* investigation

The hormones of the pituitary and parathyroid have not yet been labeled or studied with radioisotopes Except for those of the posterior pituitary, these are complex proteins, and their investigation will consequently provide greater difficulties than those associated with hormones of simpler chemical composition

will have to be concentrated. Similarly, radiochlorine, Cl^{38} (37 minute half-life), has been used for brief experiments only, but long-lived Cl^{36} (about 10^6 year half-life) is now available from the pile. Studies by Noonan *et al* (172) and Fenn *et al.* (60) with K^{42} and by Manery and Haege (152) with Cl^{36} have established the rate of penetration of ions of these elements into various tissues and organs

The penetration of Na and K through the cell wall of erythrocytes in various species have been shown with tracers by a number of workers, e g, Kurnick (132), Dean *et al* (35), Levi (143). This has necessitated a theory of selective penetrability in place of one of impermeability to explain the higher concentrations of Na in the plasma and K in the corpuscles

Bone and Teeth

The mineral metabolism of bone and teeth has been studied with radioisotopes of all of the elements regarded as essential in the inorganic parts thereof with the exception of $_8O$ and $_{12}Mg$, for which no useful species exist The elements involved have been $_6C$, $_{11}Na$, $_{15}P$, and $_{20}Ca$ In addition, studies on $_9F$ and especially $_{38}Sr$ have also been carried out Hevesy (112) has reviewed most of these studies in detail, and only a few are given here as illustrative examples

The first biologic experiment reported for a radioisotope was the study of Chiewitz and Hevesy (23) on the deposition of P^{32}, adminministered as phosphate, in the rat skeleton, this occurs in the adult animal as well as the young Succeeding studies have established the fact that in both bone and teeth there is a constant, though slow turnover of phosphate *in vivo*, even in the enamel of teeth, which completely lacks a blood supply (114) In fact, Falkenheim *et al* (58) have recently shown that powdered skeletal substance *in vitro* absorbs phosphate in decreasing order, as follows: bone, dentine, and enamel The relative lability of the skeletal phosphate was a surprising discovery, as it had earlier been generally assumed that the inorganic part of bone and teeth were essentially inert in the adult organism Long-term experiments by Hevesy *et al.* (115) showed that in the rabbit tibia 11 per cent of the epiphysis and 3 per cent of the diaphysis were rejuvenated in 9 days, and 29 per cent and 7 per cent, respectively, in 50 days Even the tip of the

found that potassium and rubidium ions can cross cell boundaries from blood into tissues faster than other electrolytes

Although rubidium is not a normal element in the metabolism of vertebrates, its close similarity as an electrolyte to potassium is not surprising in view of their closely related chemical properties A notable exception to the more rapid penetration of potassium is the rate of entrance of iodine into the thyroid gland, a phenomenon which is discussed in more detail in a later subsection. Thus, although iodine is known to disappear from the plasma faster than all normal electrolytes except potassium and phosphate, its rate of increase in the cerebrospinal fluid has been found by Greenberg *et al* (77) to be the slowest of the seven ions studied by them They did not, however, include chloride ion in their experiments

Quimby (183,184) has recently reviewed the use of radiosodium in biology and medicine. Work with short-lived radiosodium Na^{24} (14.8 hour half-life), has had particular significance in casting light on such important problems as the permeability of the placenta to sodium (66), the mechanism of shock (62), the exchange of sodium between the intestinal lumen and the body (230,231), and the calculation of the sodium space, i e., the extracellular fluid space (61, 122,165)

Most of this work has been done with short-lived Na^{24} and has thus been in the nature of short-term experiments However, a much longer-lived radioisotope of sodium exists—Na^{22} (3 year half-life), and although its use has been limited to date, it will unquestionably find important application in the study of long-term sodium turnover Reaser and Burch (187) have already used it to demonstrate the dynamics of sodium retention in congestive heart disease and have shown that in this condition excretion may be depressed as low as 1/50 of normal Cuthbertson and Greenberg (33) have demonstrated with Na^{22} the action of dietary chloride deficiency in increasing the sodium space of the rat Radiosodium has also found use as a diagnostic tool in the study of circulatory disease; this will be discussed in the section on therapy and diagnosis

Radiopotassium, K^{42}, has been used only for short-term experiments by reason of its short half-life (12 4 hours) For application in long-term studies the rarer naturally occurring isotopes, K^{40} (radioactive with a half-life of around 10^9 years) and K^{41} (stable)

of great interest to establish whether fluorine is a micronutrient dietary requirement Radiofluorine may well aid the ultimate answering of this question.

The uptake of other elements in bone—the lanthanide and actinide rare earth, radium, etc.—is considered in the section on pharmacologic and toxicologic studies.

Trace Elements

Of the so-called "trace" elements—those required in small or even minute amounts in nutrition—the roles of iron and iodine are the best understood Their metabolism has been extensively studied with tracers. In addition, there are a number of elements for the essential role of which definite or strong evidence exist: $_{25}$Mn, $_{27}$Co, $_{29}$Cu, $_{30}$Zn, and possibly $_{35}$Br

Iron. The tracer study of iron metabolism has been recently reviewed in detail by Hahn (86) and by Hevesy (112). There are two radioactive species available—Fe^{55} (5 year half-life) and Fe^{59} (47 day half-life); of these, the latter has been employed in the majority of studies.

Hahn *et al.* (87), by means of the iron label, conclusively showed a remarkable metabolic property of iron, namely, that the body controls the level of its iron stores by regulating absorption rather than by excretion The unusual mechanism that the absorption of iron involves has been discussed by Hahn and co-workers (86,90) In the intestinal mucosa is to be found the protein apoferritin or ferritin which is capable of combining stoichiometrically with 23 per cent of its weight of iron The liver and intestine contains labile iron pools of iron bound in ferritin. These are in equilibrium with the plasma-bound iron When blood is lost the increased demands of the marrow take iron from the blood, which in turn takes up iron from the labile stores in the liver and intestine The formation of ferritin iron from inorganic and hemoglobin iron has been followed by Hahn *et al* (90) and by Granick and Hahn (75) with Fe^{59} Greenberg and Wintrobe (80) have calculated the labile iron pool as about 133 mg. in man

Hemoglobin and plasma iron do not undergo exchange during the life of the red cell (88). Thus the turnover of iron in erythrocyte hemoglobin is very slow. When erythrocytes disintegrate as a

incisor enamel, which was formed before the administration of labeled phosphate, underwent about 7 per cent rejuvenation in 50 days.

There have been a number of studies on the effect of vitamin D excess and insufficiency on the phosphate turnover of bone. For example, Shimotori and Morgan (204) observed that a large dose of vitamin D acted more to increase phosphate turnover in the bone than to mobilize phosphate from the gut.

The turnover of bone calcium has been studied with Ca^{45}, and indirectly with Sr^{89}, since strontium, as a homologue of calcium, behaves much the same in bone metabolism. The uptake of these elements is relatively much greater in bone, as compared to muscle, than is that of phosphorus, but this is to be explained on the basis of a comparatively low calcium content in soft tissues rather than a higher calcium turnover in skeletal structures (175,176). Campbell and Greenberg (19) have shown that, toward the end of 3 days after administration of radiocalcium to adult rats, the labeled content of dried bone is 130 times as great as that of dried muscle Greenberg (76) has also studied the effect of vitamin D on the healing of bone fractures and found that it accelerates calcium and strontium absorption from the intestine and directly increases the rate of bone mineralization

Studies with radiocarbon on the uptake of carbonate by bone have revealed an interesting difference between its pattern and that of calcium (and strontium). Bloom *et al.* (11) have recently shown with the long-lived isotope C^{14} (5,100 year half-life) that in rats it appears primarily in areas already ossified and much less in regions of recent bone mineralization After 16 weeks the C^{14} content of this part of the bone appeared by radioautography to be as great as after 3 days This has considerable significance as regards the possible therapeutic use of C^{14}

Studies on fluorine metabolism with radiofluorine are limited by the short half-life of the best available species, F^{18} (112 minutes). Nevertheless, it has been possible to demonstrate the selective uptake of fluorine by bone *in vivo* (233), and its ready absorption by powdered bone *in vitro* (232). In view of the known effect of fluoride on teeth in increasing resistance to caries, the exact biologic role of fluorine has considerable practical significance. It would be

and Chaikoff (218) have reported evidence that the circulating thyroid hormone is thyroxine.

Hamilton and Soley (101) were the first to study the excretion of a physiologic dose of radioiodine. They found that within less than a day 37 per cent of the dose had been excreted in the urine and 17 per cent in the feces A number of workers have concluded that the amount of radioiodine in the urine after a tracer dose can be used as an index of thyroid activity (21,108,110,163,237)

Perlman *et al.* (179) made the interesting observation that bromine is concentrated in the thyroid although to a lesser degree than iodine; and Hamilton and Soley (101) have found that astatine is taken up as strongly as iodine. Thus the behavior of these two iodine homologues is metabolically similar to that of iodine.

Other Trace Elements. Work with radioisotopes on manganese, cobalt, copper, and zinc have been in the nature of preliminary studies It is to be expected, however, that understanding the metabolic role of these elements will be assisted, if not made possible, by the use of tracers The trace elements appear to act, at least in part, as enzyme catalysts Comar (26) has recently reviewed the use of isotopes in the study of the metabolism of the micronutrient elements other than iron and iodine

Greenberg *et al* (78) have studied the excretion of manganese and cobalt by using Mn^{54} and a mixture of Co^{56} and Co^{58} Manganese is excreted almost entirely in the feces, whereas the urine is the chief pathway for cobalt Both elements contrast sharply with iron, which is excreted scarcely at all Born *et al* (13) have used the short-lived isotope Mn^{56} for studying the concentration of manganese in various organs of the rat after intravenous injection At the end of 6 hours the liver had the highest concentration, followed by the thymus, kidney, and adrenals

Comar and Davis (27,28) have studied the fate of cobalt injected into the jugular vein of cattle Comparisons between swine and rabbits suggested no species difference in cobalt metabolism

Labeled colloidal manganese dioxide according to Hahn, Sheppard, and associates (91,201,203) is taken up in the lymphoid reticulo-endothelium

Copper uptake has been studied with Cu^{64} by several workers (195,196,243) The liver concentrates the highest proportion, in

result of aging or trauma, the labeled iron from the liberated hemoglobin is used almost immediately in the formation of new corpuscles This renders the use of the iron label to determine the life of the red cell rather difficult However, Hawkins and Hahn (106) studied the activity of the red cell hemoglobin in a dog given acetylphenylhydrazine followed by radioiron, and found that the red cell turnover period was approximately 70 days

Labeled erythrocytes have been used to determine the circulating red cell volume in dogs (89) and humans (70,161), the distribution of red cells in dogs (69), and the survival of preserved human erythrocytes stored as whole blood or by resuspension after removal of the plasma (68,71,72,192).

Iodine Iodine metabolism as studied with three radioactive species, I^{128} (25 minute half-life), I^{130} (12 6 hour half-life), and I^{131} (8 day half-life), has been recently reviewed by Leblond (141) and by Hevesy (112) The intimate relation of this element to the physiology of the thyroid and thus to the maintenance of the normal basal metabolic rate has long been known

The many studies with radioiodine can be little more than touched upon here The fact that only trace amounts of iodine are needed in normal metabolism has made radioisotopes of inestimable value in problems of thyroid physiology, as it is possible to administer readily measurable doses sufficiently minute that no derangement in the normal activity of the thyroid ensues Perlman *et al* (178) in 1941 showed that only 7 per cent of a 0 03 mg dose of a labeled iodine was concentrated in the rat thyroid, whereas 65 per cent of a tracer dose of negligible weight was picked up Leblond *et al.* (142) found, in dogs with a high iodine level in the blood, that 75 per cent of the radioactivity picked up 30 minutes after the injection of a minute dose of radioiodine was present as iodide and the rest was in the thyroglobulin Twenty-four hours later, however, well over half of the iodine present was organically bound and could be identified in the thyroxine and diiodotyrosine of the thyroid

Radioiodine has provided an answer to the question of the biochemical steps involved in the incorporation of iodide into the thyroid hormone (141), these are now known to be the iodination of tyrosine molecules to diiodotyrosine followed by the oxidative coupling of diiodotyrosine molecules to form thyroxine Recently Taurog

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Zinc injected in trace amounts into mice and dogs by Sheline *et al.* (200) and Montgomery *et al.* (164) was followed by means of isotope Zn^{65}. Most was excreted by way of the feces. The pancreas concentrated zinc to the highest degree, followed by the liver and kidneys. Müller (170) used the short-lived isotope Zn^{63} to label zinc suspended in a pectin colloid and found that he was able to localize zinc injected subcutaneously or intramuscularly in this vehicle in the region of inoculation. By this means he was able to get localized radiation effects on malignant tissues

After injection of Zn^{65}, the isotope is present in the red and white blood cells of man and dog, and has been found to persist in the cells for as long as eight months (227a).

PHARMACOLOGIC AND TOXICOLOGIC STUDIES

A number of elements not thought to be required in the normal body economy are of importance in pharmacology, particularly, although not exclusively, by reason of their usefulness in the treatment of spirochetal, protozoan, and helminthic diseases As a rule, although poisonous to the human, they are, when appropriate, more poisonous to the parasites These elements include ${}_{33}As$, ${}_{51}Sb$, ${}_{80}Hg$, and ${}_{83}Bi$, and are used in both inorganic and organic combinations. Such elements as ${}_{47}Ag$ and ${}_{79}Au$ have also found an important place in therapy. Of these six, only ${}_{47}Ag$ and ${}_{80}Hg$ have not as yet been studied with tracers in mammalian metabolism.

The study of organic pharmaceuticals labeled with C^{14} or H^3 has not yet begun There have been very few tracer studies on the deranged metabolic processes induced by toxic agents, either by labeling the agents themselves or by introducing labeled normal metabolites A few studies have been carried out on the fate, in the mammal, of toxic amounts of certain substances Unquestionably there are important possibilities for the application of isotopes to problems in pharmacology and toxicology—particularly in elucidating the rationale for the use of many empirically derived therapeutic agents.

One phase of what may be considered toxicology has received a fair amount of attention, for recently there has been considerable

interest in the study of the absorption, distribution, and excretion

been studied in this way; these are listed in Table I. In the case of plutonium and the elements occurring in fission, these studies have considerable potential importance for an understanding of some of the public health hazards of nuclear energy installations (98).

Elements Important in Pharmacology

Labeled sodium arsenate injected into mice in amounts negligible by weight is found after 6 hours in highest concentration in the kidney and in decreasing amounts in the blood, lungs, spleen and liver, salivary glands, gonads, and brain (12). There have been other studies on the injection of varying amounts of labeled arsenic. When tracer-free arsenic as sodium arsenate is administered to cats or rats, 50 per cent of the dose is found in the erythrocytes, only 3 per cent in the skeleton, and less than 2 per cent in the other organs at the end of 4 days.

The selective affinity of the filarial parasite *Litomosoides carinii* of the cotton rat for sodium arsenite has been shown by Lawton *et al* (139), 1 6 mg of the labeled drug administered to infected animals resulted in a highest concentration of radioactivity in the kidney, followed closely by the liver and the parasite. This result suggests the manner in which radioisotopes may aid in establishing the rationale of drug action

Work of Lowry *et al* (151) with labeled arsenite has indicated that there is little evidence that arsenic can replace its homologue phosphorus in the tissues. They found, however, that arsenic concentrated in erythrocytes appears bound to the hemoglobin molecule

Distribution studies on antimony, using the isotope Sb^{124} (60 day half-life), have been carried out with labeled tartar emetic (6,16, 32), sodium antimonyl xylitol (16,32), and stibine (208). The most interesting observation has been that, in dogs infected with the filaria, *Dirofilaria immitis*, and injected with Sb^{124} labeled sodium antimonyl xylitol (32), the isotope was concentrated in the parasite more than in any host tissue except the thyroid and liver.

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Bismuth distribution in mammals was one of the earliest problems studied with a radioisotope labeling the stable form of an element. In 1924, Christiansen *et al* (25), using Bi^{210} (Ra E) (5 day half-life), were able to show that about 70 per cent was excreted in the urine and the rest in the feces, a behavior different from that of lead

Gold has been much used in the therapy of rheumatoid arthritis General distribution studies using Au^{198} (2 7 day half-life) have shown that colloidal gold and various gold salts concentrate principally in the kidney, spleen, liver, adrenals, and bone marrow, but that appreciable amounts of gold sodium thiosulfate get into the synovial membrane, particularly when inflamed (10). Again, this time in the case of chrysotherapy, the use of a radioisotope suggests that the rationale of a drug may be worked out with the help of tracer methods.

Radium and Radon

The literature on radium and radon is voluminous, Stoklasa and Penkava (213) have reviewed it up to 1933 Although it is true that the tracer technic as a concept in biology originated with Hevesy, who was the first (111) deliberately to label a normally stable element ($_{82}Pb$) with a radioactive species (Pb^{210} or RaD), the distribution of radium was earlier studied not only with the major isotope Ra^{226}, but also with Ra^{224} (ThX) (17). Radium and radon, with their decay products, have had wide therapeutic application Early workers administered radium internally (181,181a), but when the striking toxic effects of minute amounts were reported (158), this use was abandoned Radium, like its homologues, calcium and strontium, localizes in bone (185,241) where it may induce sarcomas as a result of its chronic radioactivity (53) Studies with Ra^{224} (241) have shown that it is also taken up very strikingly in the thyroid

Radon, being a gas, can be inhaled and its distribution followed (145). It can be detected in the expired air of mammals that have been given intravenous doses of any of the heavy elements which decay to one of its isotopes For example, Stenstrom (212) has demonstrated Ra^{220} or thoron (Tn) in the breath of rabbits that have been given intravenous colloidal thorium dioxide (thorotrast), which is taken up in the reticulo-endothelial system

Lange and Evans (134) have found that radon administered in ointments is taken up appreciably through the intact skin and to an even greater extent through open wounds.

Elements in Fission and the Actinides

The new exploitation of nuclear energy, depending on the fission process in certain isotopes of uranium and plutonium, has awakened interest in a large number of elements which hitherto have had little or no biologic importance The large amounts of uranium, plutonium, and possibly thorium that must go into the piles means that contamination of personnel must be guarded against Especially important are the fission products themselves (isotopes of elements from $_{30}$Zn to $_{63}$Eu), with their intense radioactivity and the unprecedented concentrations of relatively short-lived species Thus, the recent works on the absorption, distribution, and excretion of most of the actinides, or new rare earth elements, of which 8 are known ($_{89}$Ac through $_{96}$Cm) and of a number of important fission products have particular significance. Hamilton (98) has studied the fate of tracer amounts of $_{90}$Th, $_{91}$Pa, $_{93}$Np, $_{94}$Pu, $_{95}$Am, and $_{96}$Cm among the actinides, and of $_{38}$Sr, $_{39}$Y, $_{40}$Zr, $_{41}$Cb, $_{44}$Ru, $_{52}$Te, $_{54}$Xe, $_{55}$Cs, $_{56}$Ba, $_{57}$La, $_{58}$Ce, $_{59}$Pr, and $_{61}$Pm among the elements occurring in fission

Certain of the elements occurring in fission have been shown by Copp *et al* (31) and Hamilton (98) to localize in bone after intravenous administration in rats The rare earth elements, as well as yttrium, zirconium, and columbium, are particularly striking in this regard These elements are not absorbed in appreciable amounts from the digestive tract, but when administered as aerosols, they remain for considerable periods in the lung tissue In the case of strontium, uptake either from the digestive tract or lung tissue is rapid, in fact, within 4 hours 60 per cent of the Sr^{89} originally deposited in the lung has been found in the bone

Lead and Polonium

In addition to the toxic elements already covered in this section there are two others—lead and polonium—that have not been considered Of these, only lead has had practical importance as a toxic agent

The distribution in the mammal of lead labeled with Pb^{210}(RaD) was early studied by Christiansen *et al.* (24); Behrens (8) followed shortly with the application of Pb^{212} (ThB). Recent studies by Wolf *et al.* (241) using Pb^{212} have shown that the activities of organs in the rat found after subcutaneous injection of 10 mg. of labeled $PbNO_3$ is only 1/100 to 1/10 of the activities found after injection of the isotope alone. The kidneys concentrated the most isotope, and the relative concentrations in the various organs was almost independent of the amount of lead administered.

Mortenson (169) has studied the uptake of tetraethyllead vapor inhaled by rats; 16 to 20 per cent of the compound reaching the lungs was absorbed, and an uptake of 11 4 mg. per kilogram of body weight was found to be fatal.

The distribution of intravenously administered polonium was investigated by Lacassagne and Lattes (133) in 1924 They found that it was concentrated particularly in the cells of the reticulo-endothelial system—spleen, lymphoid tissue, thymus, bone marrow, etc These workers were the first to employ the radioautographic technic.

RESPIRATORY EXCHANGE

Radioisotopes of the noble gases have been of particular value in the study of respiratory exchange in man and other mammals. The isotopes used have been A^{41}, $Kr^{79, 81}$, and Xe^{127} (120); in addition, the short-lived radioisotope of nitrogen, N^{13}, has found some application (120).

Cook and Sears (29) have studied the effects of temperature and various drugs on the rate of uptake of radiokrypton in the dog. Using the *in vivo* method of measurement, they applied a Geiger-Müller counter to the hind foot shielded with lead Increases in temperature hastened, and decreases depressed, uptake Adrenalin, histamine, and dexedrine depressed the rate of uptake, whereas the xanthines facilitated it

Jones (120) has analyzed the curve obtained in measuring the activity of expired air from subjects eliminating previously inspired radioactive inert gases and has been able to develop from such data a concept of groups of organs with different "perfusion factors," that is, volumes of blood flowing through a unit of tissue in a unit time

In general, the rate of uptake and elimination of the noble gases is believed to parallel the uptake and elimination of nitrogen. Thus, the use of these isotopes has been of importance in studying the physiology of bends, or caisson disease (120).

NONMETABOLIC PHYSIOLOGIC STUDIES

Labeling a variety of materials and following their fate in the body is readily achieved with radioisotopes Such labeled substances may be used as adjuncts to the study of important physiologic problems. For example, colloidal chromic phosphate is metabolically inert and has been found by Jones *et al.* (121) to localize largely in the liver and spleen This has provided an ingenious method of measuring the rate of blood flow in the liver (36).

The labeling of bacteria has been attempted with P^{32} by Ely (43) for several species, including *Escherichia coli* and *Staphylococcus aureus,* and by Hevesy *et al* (114a) for tubercle bacilli. Ely found that after intravenous inoculation, the highest activity was to be observed in the liver and intestine. Hevesy *et al.* inoculated their labeled bacilli subcutaneously in the guinea pig and found that after 4 hours the lymph glands had the highest activity, followed by spleen and liver Such experiments offer difficulties in interpretation because of the metabolic exchange between bacteria and host

These examples serve to indicate the variety of nonmetabolic problems to which isotopic indicators may be applied

Radioisotopes in Therapy and Diagnosis

The therapeutic application of radioisotopes followed the discovery of natural radioactivity by only a few years. Since Danlos and Bloch (34) in 1901 first employed Ra^{226}, the major radium isotope (which historically and popularly has been the "radium" of medicine), the science of radium therapy has become one of the most important in radiology Both Ra^{226} and Ra^{228} or mesothorium 1 ($MsTh_1$) (often referred to simply as "mesothorium"), have been used clinically, although today it is exclusively the former (together with its decay products) that has general application Rn^{222}, the major radon isotope, (with its decay products) is also widely used

The natural radioactive isotopes and their decay products have been used extensively in therapy. Treatment of neoplasms, superficial skin lesions, dermatoses, etc., with radium and radon has been adequately described in numerous books on radiation therapy.

The discovery of artificial radioactivity in 1933 immediately opened a new domain for the therapeutic exploitation of radioactive phenomena. The possibilities offered in this connection by the new atomic species were recognized almost immediately. Hamilton and and Stone (104), in 1936, treated a few cases of leukemia with intravenously administered Na^{24}, but the dosages used were not sufficient to produce any clear-cut effect in the time during which the patients were followed The cases were reported in the literature in 1937. In 1936, J H Lawrence also began the use of P^{32} in the treatment of leukemia, and, by 1939 when he and associates (137) published their first case histories, the usefulness of this isotope was apparent

✓In 1942, the successful use of radioiodine, I^{130}, in the treatment of hyperthyroidism was simultaneously announced by Hamilton and Lawrence (99) and by Hertz and Roberts (109), and since then it has become apparent that I^{130} and I^{131} are excellent therapeutic aids in the treatment of this disease and also of the rare thyroid malignant tumors that localize iodine (198,199). In fact, radioiodine is the most satisfactory radioelement as yet used for therapeutic purposes

Radiophosphorus and iodine have been of value in therapy because of the selective localization of the isotopes as a result of their normal metabolism Iodine is concentrated in the thyroid gland to an extent many hundreds of times greater than in the rest of the body, phosphorus, because of its turnover in rapidly growing tissue, is concentrated to some extent in the nucleoprotein of the bone marrow and liver cells as well as in bone

It is apparent that therapy with the radioisotopes is dependent upon more than a limited amount of localization In the treatment of the hematologic disorders amenable to P^{32} much care must be exercised because of the danger of radiation damage to the normal cells of the bone marrow Treatment of the lymphomas with radiophosphorus is more or less unsatisfactory because the uptake of the

isotope in the tumor cell is only about twice that of the normal cells surrounding it. An isotope, to be of value therapeuticaully, should have at least a tenfold concentration in the tissue that is to be irradiated over that in normal tissue

Within the last five years radiosodium (Na^{24}) has been re-evaluated favorably as a therapeutic agent (55,56). Thus, at the present time, the isotopes P^{32}, I^{130}, I^{131}, and Na^{24} are the only artificial species with a well-established clinical role. However, there can be little doubt that within a few years isotopes of other elements will be utilized as therapeutic agents

Since 1942 isotopes of manganese (Mn^{52}), cobalt (Co^{60}), zinc (Zn^{63}), strontium (Sr^{89}), yttrium (Y^{91}), zirconium (Zr^{95}), columbium (Cb^{95}), and gold (Au^{198}) have been reported or suggested on experimental grounds as radiotherapeutic tools. In Table III are listed the isotopes that have been used or suggested in therapy.

Recently, Hahn and Sheppard (92) have gone over the approximately 450 isotopes known in 1944 and selected those that appear to offer the most promising features for therapeutic application. They set up certain criteria of therapeutic usefulness, as follows

(1) The isotopes should be pure and free of contaminants
(2) The "half-life" should be under 10 days and over 2 days
(3) The physical and chemical properties must be known and the biologic behavior understood.
(4) Localization and selectivity in deposition are necessary

On the basis of the foregoing criteria, they were able to list only 18 isotopes, and of these several do not strictly fulfill all the demands of practical usefulness For example, P^{32} is to be regarded as undesirable because of an overlong half-life It is true that the control of dosage for P^{32} is more difficult than for short-lived isotopes, yet the use of this species in the treatment of chronic leukemia and polycythemia for over 10 years has given results as good as, and possibly slightly better, than those with X-ray, and to date, there has been no evidence of the induction of malignancy in bone or soft tissues from the amounts employed in therapy However, the substitution of a shorter-lived isotope for P^{32} may well follow the coming availability of many previously unfamiliar species Moreover,

Hahn and Sheppard's criteria may well justify the abandonment of such species as Sr^{89}, Y^{91}, Zr^{95}, and Cb^{95} as therapeutic possibilities, despite their ready availability from pile sources, because of the fact that their half-lives are between 1 and 2 months

TABLE III

Isotopes Used or Suggested in Therapy

Element	Isotope	Disease treated	Result
Hydrogen*	H^1 (as proton beam)	(Suggested for deep cancer)	?
Beryllium	Be^9 (natural element)	Various malignancies	Palliative
Sodium	Na^{24}	Chronic leukemia	Palliative
		Polycythemia vera	"
Phosphorus	P^{32}	Leukemia	Palliative
		Polycythemia vera	"
		Basal-cell carcinoma	Curative
		Warts, etc	"
Manganese	Mn^{52}	Local irradiation of lymph nodes	Palliative
Cobalt*	Co^{60}	(Suggested as substitute for radium)	?
Zinc	Zn^{65}	Uterine carcinoma	?
Strontium	Sr^{89}	Bone tumors	Poor
Yttrium	Y^{90}	Chronic leukemia	Palliative
	Y^{91}	" "	"
Zirconium	Zr^{95}	" "	"
Columbium	Cb^{95}	" "	"
Iodine	I^{130}	*Hyperthyroidism*	Curative
	I^{131}	Carcinoma of thyroid	Palliative
Gold	Au^{198}	Leukemia	"
		Local tumors	"
Radon (and decay products)	Rn^{222}	Carcinomas, dermatoses, etc.	Palliative *and curative*
Radium (and decay products)	Ra^{226}	"	"
	Ra^{228} ($MsTh_1$)	"	"

* Elements and isotopes not yet applied

The use of labeled colloidal preparations that localize selectively in the reticulo-endothelial system was suggested in 1944 by the work on anhydrous chromic phosphate by Jones *et al.* (121). A number of workers have since explored various colloidal preparations. These offer, perhaps, one of the most promising possibilities for future development in isotope therapy because of the localization possible with such compounds

PHOSPHORUS

Diagnosis

P^{32} has been suggested as a useful diagnostic agent in breast tumors by Low-Beer and co-workers (147,149) Small doses of P^{32}, when administered to women with suspicious breast lesions, were picked up in greater amounts (about 25 per cent) by malignant tumors than by the normal surrounding tissue. Benign lesions showed a small percentage of uptake of radiophosphorus Similar results have been obtained by us (65a) and others in superficially located carcinomas. This method should prove of value as an aid to diagnosis in suspected cancerous lesions; it seems unlikely, however, that it can ever take the place of a biopsy.

Red cells labeled with P^{32} have been used in blood volume studies (84) Nylin and Malm (173) utilized erythrocytes labeled with P^{32} to determine the causes of the prolonged circulation time in cardiac decompensation

Therapy

Radioactive phosphorus was the first artificial radioactive isotope successfully used in therapy Since the original report (137), considerable information concerning the therapeutic value of P^{32} has accumulated and an excellent comprehensive review has recently been published (190). Radiophosphorus, when administered orally or intravenously, is somewhat selectively concentrated by rapidly metabolizing tissues (30,235), such as bone marrow, liver, and tumors. As noted by Reinhard *et al* (190), this selectivity is dependent upon. "(*1*) The total amount of phosphorus in exchangeable form in the tissue, (*2*) the rate of turnover of phosphorus by the tissue, and (*3*) the rate at which new tissue is formed"

Lawrence and co-workers (138) showed that the radiophosphorus content of the lymph nodes and spleens of leukemic mice was twice that of similar organs of normal animals Increase in the P^{32} content of the nucleoproteins of lymph nodes, spleen, and tumor tissue of leukemic mice was demonstrated by Tuttle, Erf, and Lawrence (226,227), and Marshak (159) demonstrated the increased P^{32} content of the nuclei of malignant cells as compared with normal cells

Increased concentration of radiophosphorus in the spleen, liver,

bone marrow, and lymph nodes of leukemic patients has been demonstrated (44,47,48,235). Variable findings in uptake of P^{32} in the lymph nodes of the case of other lymphoblastomas and malignant tumors have been observed (127–130).

Thus neoplastic and leukemic tissues concentrate radiophosphorus to some extent, at least. The limiting factor in the use of P^{32} as a therapeutic agent is the deposition of the phosphorus in normal as well as malignant cells, so that the amount of P^{32} administered must be gauged carefully in order to avoid toxic effects on normal tissue.

P^{32}, carrier-free as obtained from Oak Ridge, is standardized, made isotonic with sodium chloride, and, after neutralization with sodium hydroxide, is autoclaved; 0.5–1.0 cc. is then injected intraperitoneally into mice. After 48 hours, if no reaction has occurred, the material is ready to use.

Radioactive phosphorus, when administered by mouth, is excreted in the urine and feces (49). About 25 to 50 per cent of an oral dose is excreted in 4 to 6 days in both normal and leukemic patients; intravenous administration of P^{32}, however, results in an excretion of only 5 to 25 per cent of the isotope, mostly in the urine (112)

According to many investigators (45,46,94,135,189,190), radioactive phosphorus is the treatment of choice in polycythemia vera. The dosage employed must be individualized. A total dose of 5 millicuries (mc.) of P^{32} intravenously is usually sufficient to produce a hematologic remission It is our policy (237) to administer 3 mc of the isotope intravenously, following a complete hematologic examination. One month later, a sternal marrow aspiration and the peripheral blood findings are again analyzed If the bone marrow is still as active as it was before treatment and no peripheral blood change has occurred, another dose of 2 to 3 mc. is injected A change in either the marrow or blood, usually a reduction in the total number of nucleated cells in the bone marrow and a drop in the white count, indicates that further therapy with P^{32} should be postponed Figures 1 and 2 represent long-term hematologic remissions in 2 cases of polycythemia vera treated with moderate doses of radiophosphorus Variations in dosage required to produce remissions makes extreme caution in the use of the isotope

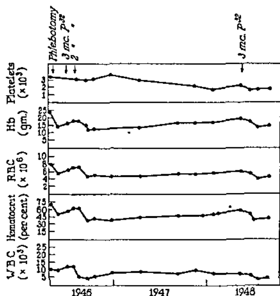


Fig 1 Polycythemia vera treated with small doses of P^{32}. Note long remission lasting almost 2 years and subsequent response to 3 mc of P^{32}

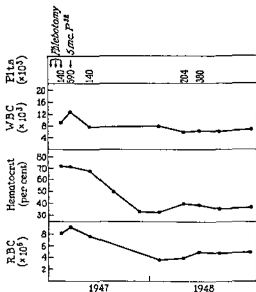


Fig 2 Polycythemia vera treated with P^{32} Note maximum response after 6 months and then a levelling off of the blood elements.

mandatory. In one of our polycythemia patients, 5 mc. of P^{32} caused an extreme depression of the marrow, necessitating blood transfusions to combat the temporary bone marrow aplasia On the other hand, large doses of the order of 10 to 20 mc. are occasionally necessary to promote a remission in polycythemia vera. Complications of P^{32} therapy may be anemia, leukopenia, and thrombopenia (107).

As seen in Figure 2, however, the radiation from P^{32} may take as long as 6 months to produce the maximum depression of the blood elements; hence, after the 5 mc. dose of radiophosphorus has been injected, an interval of 4 to 5 months should be permitted to elapse before further therapy is administered. Remissions in polycythemia following P^{32} may last from 6 months to several years

The treatment of the chronic leukemias (myeloid and lymphoid) with radioactive phosphorus is as satisfactory as the therapy of these diseases with X-ray and other chemotherapeutic agents (119, 136,189,190,236). Hematologic and symptomatic remissions can be maintained for long periods It has been stated that one of the advantages of P^{32} over x-ray therapy in the leukemias is the absence of radiation sickness following radiophosphorus.

Small doses of P^{32} (0 5–1 mc) are administered intravenously once or twice weekly for a total of 4 to 6 mc The peripheral blood is followed carefully and symptoms evaluated. When a satisfactory reduction in white blood cells is achieved, no further P^{32} therapy is given for 4 to 6 months A combination of P^{32} and x-ray therapy may be more effective than either one alone; and x-ray is probably to be preferred to P^{32} when local reduction of lymph nodes or spleen is desired

The acute leukemias, lymphoblastomas, and related diseases are not usually affected by radioactive phosphorus P^{32} may be somewhat helpful in multiple myeloma (138a) and serve as an adjuvant in follicular lymphoblastoma when treated with x-rays. Bayrd and Hall (7) have recently reported an unusual remission in a case of acute plasma-cell leukemia following P^{32} therapy.

Low-Beer (146,148) has described a technic for local treatment of some skin lesions with blotting paper soaked in measured amounts of P^{32} solution Basal-cell carcinomas, warts, and hemangiomas have been successfully treated with this method

RADIOIODINE

Diagnosis

The use of radioactive iodine provides a particularly good method for determining the status of iodine metabolism in the human organism. Chemical studies involving estimation of iodine concentration in tissues or in blood have been difficult, since the amounts involved were of the order of a few micrograms. In order to trace the fate of ingested iodine, doses of the order of 1 or more milligrams had to be given, and it was never certain whether this dose altered the iodine metabolism by its own action With radioactive iodine, I^{131}, which has a half-life of 8 days, the usual tracer dose of 100 microcuries (μc) contains less than a thousandth of a microgram of iodine, well below the minimum for physiologic effect The emission of both beta and gamma rays of sufficient energy by radioiodine permits accurate assay of the urine and the blood, while external measurement of the radiation over the thyroid may be performed to estimate the amount of iodine present in the thyroid gland.

Radioactive iodine is furnished in the form of the iodide, and is usually administered by mouth in tap water The iodide is mostly absorbed in the stomach, and enters the blood stream as iodide. Less than 1 per cent of the dose is excreted in the feces, and the same amount is lost in the sweat Up to 15 per cent is distributed generally throughout the body, and the remaining 80 per cent or more is either taken up by the thyroid or excreted by the kidneys Once the iodide is in the bloodstream, there is competition between the thyroid and the kidney for its removal In the absence of previous large doses of iodide (2 mg or above), thiocyanate, or one of the thiourea class of drugs, iodide is selectively taken up by the thyroid at a rate generally in relation to the activity of the gland In hyperthyroidism, with the greater avidity of the gland for iodide, this substance will be removed from the bloodstream by the thyroid at a faster rate than in myxedema, where there is very little affinity of the thyroid for iodine, and the kidneys excrete a larger portion of it In the normal, or euthyroid, patient, the situation is somewhere between these two extremes

Since 1939, when Hamilton and Soley (100a) published their original work on this subject, many investigators have confirmed

these generalizations (126,186,209,210). In recent years, there has been an increased effort to utilize some feature of iodine metabolism as a measure of thyroid function, and thus as a diagnostic tool for disorders of the thyroid gland, such as Graves's disease and myxedema

In the simplest of these diagnostic tests, radioactive iodine is given to patients in a tracer dose of about 100 μc. Urines are collected for 2 or 3 days, either as 6, 12, or 24 hour specimens The total amount of I^{131} excreted is expressed as a proportion of the amount ingested In general, a high excretion (70 to 90%) means low avidity of the thyroid for iodine and thus hypothyroidism, while a low excretion, of the order of 30 per cent or less, signifies increased thyroid uptake and hyperthyroidism However, experience has shown that considerable scatter of data occurs in both normal and abnormal individuals, so that borderline cases are difficult to evaluate. Refinements of this technic, utilizing hourly urine excretion studies, improved the sensitivity of the method (126,237).

Keating and co-workers (126) reported excretion curves following the administration of tracer doses of radioiodine and derived a mathematical formula for calculating the collection rate of the thyroid Similar results have been obtained by us, using indwelling catheters and hourly excretion assays Thyroid uptake results derived from urinary studies are susceptible to numerous errors, however, such as the loss of iodine in the sweat and the soft tissues of the body, as well as the errors inherent in measuring the rate of excretion of any nonthreshold substance with diurnal variations, the effect of fluid ingestion, position and activity of the body, and blood flow.

Accurate measurements over the thyroid gland with a suitable detection instrument give the most direct evidence of thyroid function. The tracer dose administered is usually 100 μc of I^{131}

Figure 3 illustrates the thyroid uptake in 3 characteristic cases (normal or euthyroid, hyperthyroid, and hypothyroid) following administration of tracer doses of I^{131} Note the curve representing thyroid uptake in the hyperthyroid patient, the peak of uptake was reached very quickly, in about 3 hours, and represented about 95 per cent of the total amount ingested Cases of Graves's disease, followed for a period of weeks, have shown an effective half-life of

about 6 5 days which corresponds to a biological half-life of about 34 days. The curve representing the case of hypothyroidism is quite different; here, there is a total of only 5 per cent uptake in the gland, a maximum that is reached after 24 hours The third curve, in a normal case, shows a rise in thyroid uptake somewhere between the two extremes. The first portion of the curves is a parabola and the accumulation gradient of iodine in the thyroid, a measure of the rate of uptake, can be calculated (210,211). This direct measurement of thyroid uptake is less susceptible to variations in kidney excretion, sweat, and other deviations.

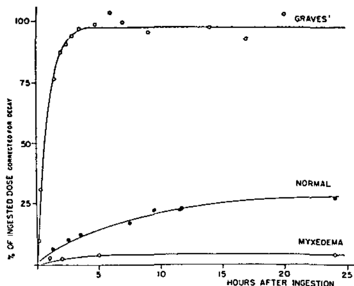


Fig 3 Radioiodine uptake in the thyroid gland of a normal, *myxedematous, and hyperthyroid patient*

No discussion of the use of radioiodine in diagnosis would be complete without mention of the use of radioautographs (97) Following a tracer dose of radioactive iodine, and after sufficient time has elapsed to permit fixation of iodine in the thyroid tissue (usually 24 to 48 hours), a microtome section of the tissue removed by biopsy or operation, when approximated to a photographic film,

produces a representation of the radioiodine-bearing portion of the section Although in the non-nodular goiter characteristic of the usual cases of Graves's disease there is diffuse and homogeneous distribution of iodine, in nodular goiter the distribution of functional thyroid tissue may be spotty, with all the activity concentrated in one or more of the adenomas (Fig. 4). Spotty distribution is also seen in Figure 5, a histologic section and autograph from a case of amyloidosis of the thyroid with thyrotoxicosis Radioautography has been used successfully in determining uptake in carcinomas of the thyroid, establishing that the tumor or the metastasis is or is not composed of functioning thyroid tissue (Fig. 6). The importance of radioautographs is well demonstrated in Figure 7. Examination of the histologic section in this case, prior to the autograph, resulted in a diagnosis of anaplastic thyroid carcinoma, probably nonfunctional; thus, treatment with large doses of radioiodine appeared valueless. The interesting positive autographs, however, indicated that localization of radioactive iodine within the tumor could be accomplished, and a therapeutic trial seemed indicated An excellent review of the value and use of radioautographs in carcinoma of the thyroid is to be found in the article by Marinelli *et al* (155).

Study of the distribution of iodine in the body, using a collimated Geiger-Müller counter, has been reported by Feitelberg and co-workers (59). Vertical and horizontal "profiles," plotted graphically, give a fairly accurate representation of the distribution of the functional thyroid tissue and permit a more accurate diagnosis to be made. The G-M shielded tube approximating the skin is moved laterally or vertically at half-inch intervals to the right and left of the midline Counts per second obtained in this way are plotted against distance from the midline Figure 8 shows a symmetrical profile obtained in a case of diffuse hyperplastic toxic goiter. Normal thyroids exhibit a similar profile, although the total uptake may be reduced In Figure 9, a preoperative and postoperative profile in a case of nontoxic adenoma of the right lobe of the thyroid is depicted Following resection of the adenoma, the peak of radiation to the right of the midline has disappeared A functional thyroid carcinoma might give a similar picture An interesting profile is in Figure 10 (Page 124) A mass at the base of the tongue, proved by

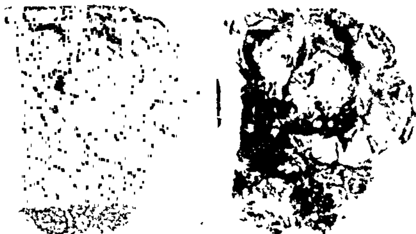


Fig 4 Radioautograph from a case of toxic nodular goiter. (Section on left, autograph on right) Note dense blackening in areas of some of the adenomas, signifying unequal distribution of the radioactive iodine.

Fig 5 Radioautographs from a case of diffuse amyloidosis of the thyroid with severe thyrotoxicosis. (Sections on left, autographs on right) Note the spotty distribution of the radioiodine in the hyperplastic areas compressed by the

Fig 6 Radioautograph from a case of anaplastic carcinoma of the thyroid (Section on left, autograph on right) The dark areas at the lower left corner and upper middle portion of the section represent the tumor No blackening appears at identical portions of the radioautograph, signifying nonfunctioning thyroid tumor tissue

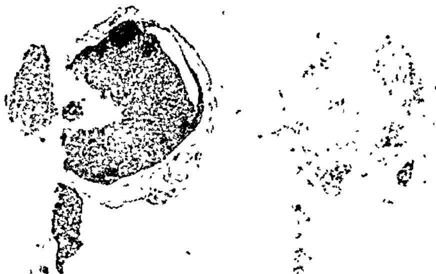


Fig 7 Radioautograph from a case of anaplastic carcinoma of the thyroid showing uptake in the tumor (Section on left, autograph on right) The dark areas in the section are sheets of carcinoma cells that appear to have no colloid, yet the autograph on the right shows blackening in parts of this carcinoma

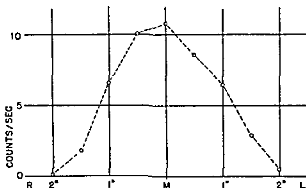


Fig 8 Horizonal profile in a case of diffuse hyperplastic toxic goiter

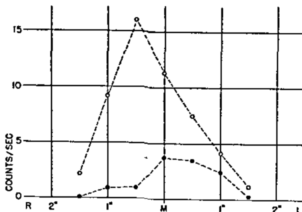


Fig 9 Horizontal profile in a case of nontoxic adenoma of the right lobe of the thyroid. Open circles, preoperative profile, closed circles, postoperative profile (59)

biopsy to be thyroid, comprised the only functional thyroid tissue in the body, with no activity detected in the neck

Carcinoma of the thyroid with functional metastases may be analyzed similarly In Figures 11–12 (p 125) are the horizontal and vertical profiles in a case of metastatic functional carcinoma of the thyroid The roentgenogram revealed evidence of metastases in the lungs in the positions noted Horizontal profiles revealed peaks of activity at the various levels

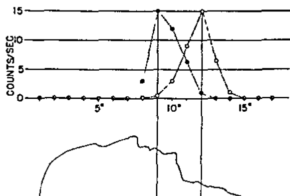


Fig. 10 Vertical profile in a case of abberrant lingual thyroid. Solid circles, record of the patient; open circles, the profile of a normal patient. Note the absence of activity in the neck, with the peak of radiation at the level of the base of the tongue (59).

Therapy

The artificial radioactive isotopes of iodine (I^{130} and I^{131}) have been the most effective of all radioisotopes used in therapy because of iodine's singular behavior in selectively concentrating in the thyroid gland to an extent about 10,000 times greater than in any other organ (141).

Hertz and Roberts (109), and Hamilton and Lawrence (99) reported on the successful treatment of hyperthyroidism with radioiodine in 1942. A few years later, reports by Hertz and Roberts (110) and Chapman and Evans (21) indicated that the mixture of radioisotopes of iodine (I^{130} and I^{131}) produced by bombardment of tellurium in the cyclotron was effective in over 80 per cent of the cases of Graves's disease; subsequent reports by Hertz (108), Soley and Miller (209), and Keating (125) noted similar results with I^{131}.

The radiations from the radioisotopes of iodine (I^{130}, 12.6 hour half-life; I^{131}, 8 0 day half-life) produce ionization in the thyroid and subsequent destruction and fibrosis, with a resultant depression of the gland function (97,160) On theoretic grounds, this form of therapy should be ideal, but fear of late malignant changes due to the radiations and of possible radiation damage to the kidneys, genital organs, and bone marrow, postponed a complete evaluation of radioiodine treatment in hyperthyroidism

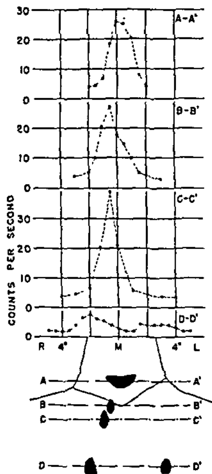


Fig 11 Horizontal profiles at various levels in a case of adenocarcinoma of the thyroid with functional metastases in the lungs. Diagram depicts location of the nodular metastases as demonstrated in the chest by roentgenogram (59) See page 123

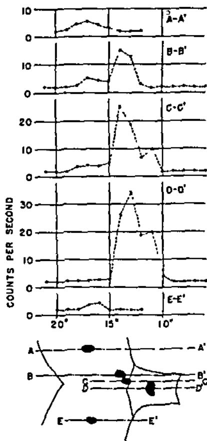


Fig 12 Vertical profiles in various planes in the same case as Figure 11 Pulmonary metastases, represented by solid circles, show uptake of I^{131} following a tracer dose Note peaks of radioactivity over lung infiltrated with tumor (59) See page 123.

About ten years have now elapsed since radioiodine was first used in the treatment of hyperthyroidism, and although only a few complete reports have appeared (21,108–110,125,163,209,238) the

experience of many investigators, including that of our laboratory, has established it on a firm basis. Previous fears have proved unjustified and, in selected cases, I^{131} seems to be the treatment of choice in hyperthyroidism. The criteria for the treatment of Graves's disease which have been used in our laboratory (237) are:

(1) All patients with diffuse hyperplastic goiters may be treated.
(2) Toxic adenomatous glands should be removed surgically. Only those cases with diffuse hyperplastic glands are treated. Patients who are poor surgical risks may, however, be treated with radioiodine.
(3) Markedly enlarged glands compressing the neck structures should be treated surgically.
(4) Where thyroid crisis is feared, radioiodine therapy should be followed by complete lugolization.

The dose of radioiodine (I^{131}) administered varies in each individual case, depending on the size of the gland, the amount of I^{131} excreted in the urine, and the effective half-life (discussed in a later subsection). One microcurie of I^{131} per gram of thyroid gives to the tissues about 160 e.r. (equivalent roentgens), assuming that the iodine remains in the gland for its total decay (238). With I^{130}, because of the shorter half-life, larger doses of the isotope were used and the cases treated successfully have received about 2,000 to 4,000 e.r. (21). In our series, a similar radiation dosage was attempted and patients at first received 2 to 4 mc. depending on the amount of iodine retained and the gland size. An appraisal of the clinical responses eventually resulted in a readjustment of the dosage, so that now, 5,000 to 10,000 e.r. (5–10 mc.) are administered to the gland, depending on the above-named factors and the degree of toxicity. A mildly toxic patient with a gland weighing 100 Gm. and excreting 25 per cent of the tracer dose is given 7 mc. of I^{131} (about 70 μc./Gm.). Larger or smaller doses may be given to more or less toxic individuals. Frequent clinical and laboratory (B.M.R. and blood cholesterol) evaluation is made. No further therapy is given for 6 to 8 weeks, subsequent dosage is administered cautiously, no more than 2 to 3 mc. being given at one time. In our experience, usually 5,000 e.r. and no more than 10,000 e.r. to the thyroid will effect a clinical remission. A clinical response is usually obtained in 2 to 3 months.

Thus far 60 patients have been treated, 52 women and 8 men, ranging in age from 20 to 80 years. Most of the patients had dif-

fuse hyperplastic glands, although 2 patients with substernal goiters and 7 with nodular thyroids were treated; the latter were all poor surgical risks. Successful remissions were obtained in all cases. Only 4 cases of hypothyroidism developed, all of a transient nature, and small doses of thyroid extract, given to control the low metabolic state, were subsequently discontinued.*

The administration of stable iodine following radioiodine to fix the radiation within the thyroid gland (110) was found to be unnecessary in the usual case of Graves's disease (21,124,238) Where a thyroid crisis is feared to be imminent, such postradioisotope treatment with Lugol's solution may be indicated.

From the experiences noted above, it is apparent that radioactive iodine in the treatment of hyperthyroidism has proved to be a valuable therapeutic agent Further experience and time are necessary to evaluate more completely the radiation hazards inherent in the use of radioactive elements.

Radioiodine uptake in carcinoma of the thyroid was originally studied with the radioautographic technic by Hamilton and coworkers (102) No deposition of radioiodine in the tumor tissue of the cases studied was noted A short time later uptake by a metastatic thyroid lesion was demonstrated by Keston *et al.* (131) and the functioning tumor was found to be well differentiated (63). Seidlin, Marinelli, and Oshry (198) reported the first case of functioning metastatic thyroid carcinoma successfully treated with radioiodine The metastatic tumor again was well differentiated, with follicles containing colloid The hyperthyroidism associated with the carcinoma responded completely to isotope therapy but the metastases, although arrested, did not disappear

The incidence of functioning thyroid carcinoma is small, being about 15 per cent of thyroid cancers (155) In a series of 19 cases studied by Marinelli *et al* (155) only one highly malignant tumor (solid alveolar-cell carcinoma) showed uptake by the radioautograph technic, the 10 cases showing radioiodine uptake were well-differentiated tumors, either metastasizing struma or follicular adenocarcinoma

Since most thyroid neoplasms are functionally inactive, successful radioisotope therapy seemed limited to a very small percen-

* *Note added in proof* Over 300 cases have been treated to this date (Feb, 1950) About 3% of the treated cases developed hypothyroidism (59a)

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tage of cases However, Seidlin and co-workers (199) have attempted to induce concentration of radioiodine in nonfunctioning thyroid tumor tissue by thyroidectomy (surgical or radiation) and/or by injections of thyrotropic hormone. This method has proved successful in their hands, and future experience with it may render many cases of thyroid carcinoma amenable to isotope therapy

However, caution in the use of these amounts of radioiodine is necessary since transient swellings, pain at the local site of the tumor, kidney damage, marrow depression, and other radiation effects may be produced (237). Patients suspected of having a thyroid neoplasm are given a tracer dose of radioactive iodine (0 1–5 0 mc.). *In vivo* tracing to determine the presence of functional tumor tissue is performed, followed by surgery and radioautographs of the neoplastic tissue. Where functional thyroid tumor is found, large doses of radioiodine (50–100 mc.) are administered at intervals until further tracer studies show no uptake. In those cases showing no pickup in the tumor, surgical or radiation thyroidectomy may be performed, followed by frequent uptake studies and massive dose isotope therapy where indicated Large doses of radioiodine (50–200 mc) have been given by many investigators without any permanent radiation effects.

The therapeutic value of radioiodine in the treatment of carcinoma of the thyroid appears to be on the increase, and future experience with the methods introduced by Seidlin should reveal the efficacy of these therapeutic procedures

SODIUM

Diagnosis

The value of radioactive sodium as a diagnostic tool (183,184) has been established Studies concerning intravascular circulation in various disease states (207) and the extravascular fluid space (61,122) have been carried out by means of radiosodium.

Kaltreiter *et al* (122) investigated the extracellular fluid space in normal and pathologic conditions, using radioactive sodium and thiocyanate simultaneously, the results obtained with Na^{24} (21% of body weight) were lower than those obtained with thiocyanate (23 5% of body weight) The concentration of Na^{24} in serous effusions was found to be the same as in the blood, the extent of serous

effusions thus could be determined by comparing the apparent "sodium space" in a given patient with that of normal subjects of the same weight

The velocity of blood flow was studied by Hubbard *et al* (118) by injecting labeled sodium chloride into one antecubital vein and detecting its arrived at the opposite hand with G-M counter. Smith and Quimby (207) used a similar technic in studying the arm-to-foot circulation time in patients with various types of peripheral vascular disease. Following the injection of the isotonic sodium chloride solution containing 100 μc. of radiosodium into an antecubital vein, the rate at which the radioactive isotope appeared in the leg was detected by a shielded G-M tube placed against the foot.

Further studies in peripheral vascular disease with labeled sodium chloride by Elkin *et al.* (42) demonstrated the difficulty in interpreting radioactive build-up curves obtained from the foot. Correlation between the clinical condition and circulation time was difficult to establish, but a useful test was developed by plotting the counting rate as equilibrium of the labeled sodium was being established between the plasma and extracellular fluid. Late equilibrium was due to arterial obstruction, arteriosclerosis, hypertension, spasm, capillary degeneration, or any combination of these factors; early equilibrium was usually associated with increased blood supply due to inflammation, or to a relaxation of the vessel walls following nerve block or sympathectomy Patients with peripheral vascular diseases having normal curves rarely showed high postoperative curves, and little symptomatic relief was obtained. These investigators concluded that the method, as used by Smith and Quimby, gave only approximate circulation time values since the variation was so great. Another method devised by Elkin and associates seems more satisfactory, radiosodium in the form of sodium chloride is injected into the muscles of the leg and the leg is elevated to impose a stress factor and accentuate the degree of vascular insufficiency. The rate of disappearance of the radioactive material is measured and it has been shown that this is proportional to the blood flow.

A new method for studying blood flow through the chambers of the heart using labeled sodium chloride was recently reported by Prinzmetal *et al.* (180) Radiosodium (Na^{24}), 100 to 200 μc, is in-

jected into an antecubital vein and its passage through the heart is recorded, using a shielded G-M counter with an ink-writing recording device. Biphasic curves representing passage through the right and left sides of the heart are obtained in normal patients Deviations are seen in cardiac enlargement, with prolonged monophasic curves being recorded.

Therapy

Radiosodium as a therapeutic agent in leukemia was first investigated by Hamilton and Stone (103,104). Failure to obtain a response was probably due to inadequate dosage, since subsequent reports (55,56,144,219) have demonstrated its effectiveness in reducing the white blood count in leukemia and in giving symptomatic improvement Radiation from radioactive sodium (Na^{24}), is not so prolonged as that from P^{32}, the half-life being only 14 8 hours It is thus easier to regulate the dosage, and treatment may be adjusted to comply with the immediate demands of the patient's symptoms and of the laboratory findings. In a comparison of the effect of radiosodium and radiophosphorus on a series of 5 cases of leukemia, Lindgren (144) found that the response to P^{32} was better than to Na^{24} According to Evans *et al.* (55,56), after a test dose of 180 μc per kilogram, larger doses of Na^{24} (10–15 mc.) in the form of sodium chloride are given. Less than 10 per cent is excreted in 24 hours Good results have been obtained in chronic lymphatic and myelogenous leukemia and in a case of polycythemia vera.

Because of the general distribution of sodium in the tissues and intracellular and extracellular fluid of the body, the radiation administered to the body by radiosodium is comparable to general body radiation and is prolonged only over a 48 hour period. However, the short half-life makes it necessary to treat the patient close to the source of supply, such as a cyclotron or the chain-reacting pile.

The place of radioactive sodium as a therapeutic agent is still obscure since the data reported is insufficient, but results similar to total body irradiation with x-rays are to be expected.

RADIOCOLLOIDS

Preliminary studies with a few radioactive colloids in the treatment of various malignant tumors and lymphomas have been made

Jones *et al.* (121) prepared insoluble chromic phosphate with P^{32} and studied its uptake and distribution in mice for many months Over 90 per cent of the radioactive colloid was selectively concentrated in the reticulo-endothelium of the liver and spleen, with the remainder distributed variously throughout the other tissues of the body. The uptake per gram of tissues in the spleen was greatest, with the liver next in order of activity. However, its use in large concentration in malignant disease of the liver or in selective splenic irradiation has not as yet been successful. The chromic phosphate is concentrated only in the normal liver tissue and not in the liver neoplasms, and thus any irradiation of a tumor is at the expense of normal hepatic parenchyma.

Equivocal results following the use of radioactive chromic phosphate in the treatment of various lymphomas in man have been reported (189)

Radioactive manganese dioxide dispersed in gelatin as a supporting colloid has been suggested by Hahn and Sheppard (91,93,201, 203). Following intravenous administration, the colloid concentrated to a great extent in the liver and also was believed to localize in the lymphoid reticulum This suggested its use in the treatment of Hodgkin's disease, chronic lymphatic leukemia, monocytic leukemia, reticulo-endotheliosis and lymphosarcoma Mn^{52} has a half-life of 6 5 days and emits both beta particles and gamma rays, but a radioactive contaminant, Mn^{54}, with a half-life of 310 days is also present, making it relatively unsuited for repeated intravenous administration

Sheppard and co-workers (202,203) have recently reported on the use of radioactive gold sols (Au^{198}) for the same purpose. Hydrated colloids of radioactive zirconium (Zr^{95}), yttrium (Y^{91}), and columbium (Cb^{95}), injected intravenously, have been utilized by Dobson and associates (37) Selective localization could be manipulated by varying the size of the colloidal particles, thus, with one method of preparation about 90 per cent was deposited in the liver and spleen, whereas decrease in the ultramicroscopic particle size resulted in an increased accumulation of the colloid in the bone marrow and an increased half-time in the blood

Colloidal uranium oxide enriched with U^{235} was shown by Tobias *et al.* (221) to have some selective localization in the liver and

spleen of mice, and *in vivo* fission of the isotope by slow neutron bombardment resulted in the release of large amounts of ionizing radiation in these tissues The biologic effect per unit of tissue ionization was greater with the fission recoils than with beta particles However, the therapeutic implications of this are obviously limited

Local infiltration of these radiocolloids in the treatment of various neoplastic tumors has been used and encouraging results have been reported (2,93,202) These colloids, when injected, remain at the site of injection. Administration of radiochromic phosphate around the periphery of spontaneous mammary adenocarcinomas in mice caused the disappearance of the tumors (2). Satisfactory treatment of a few basal-cell carcinomas of the face with this colloid has been reported (189). In addition to chromic radiophosphate, radiogold and manganese dioxide colloids have been used for this purpose (93,202). A suspension of zinc (Zn^{63}) in pectin was used in the interstitial infiltration of two cases of uterine carcinomas by Müller (170). Localization of the colloid at the region of injection occurred, and tissue reactions similar to those following radium application were observed

The ability to distribute radioactive colloids to various tissues by intravenous injection and its strict localization at the point of *infiltration when injected into or around tissues has interesting* therapeutic and diagnostic possibilities Further trial with these compounds is certainly indicated.

The distribution of the radioactive diazo dyes (dibrom trypan blue and dibrom Evans blue) has been studied in animals with inflammatory lesions and in tumor-bearing mice (167,168,222) Some selective localization in the inflammatory lesions and in the tumors has been apparent The affinity of tumor tissue for the dye, fluorescein, was recently utilized by Moore as a diagnostic test (166) Diiodofluorescein synthesized with radioiodine (I^{131}) was shown to localize in brain tumors. A shielded G-M tube mounted on a portable x-ray unit localized the tumors with a fair degree of accuracy, and correct predictions as to the operative findings were made in the 12 cases reported

OTHER ELEMENTS

The selective concentration of calcium in bone (175,176) suggested the use of radiocalcium (Ca^{45}) in the therapy of certain bone

tumors This proved impractical, however, because of the long half-life of Ca^{45} (180 days) and the difficulty in preparing a radiocalcium of high specific activity in sufficient amount. Strontium is physiologically interchangeable with calcium and the radioisotope used (Sr^{89}) has a more suitable half-life (55 days), emits an energetic beta particle, and can be easily prepared (175,176). Distribution studies in patients with malignant bone lesions revealed neoplastic accumulation of the radiostrontium in an amount only slightly in excess of that found in normal bone This absence of definitive localization in bone tumors and the deposition of the radiostrontium throughout bone has curtailed its use as a therapeutic agent; furthermore, the close proximity of the bone marrow to the penetrating beta rays of Sr^{89} makes irreversible bone marrow damage likely if sufficient isotopic concentration to destroy the neoplastic cells is achieved.

Preliminary results in the treatment of metastatic malignant disease with Sr^{89} have been reported by Pecher (176), Lawrence (135), and Low-Beer *et al* (150). Control of pain and apparent slowing down of the carcinoma were achieved

Radioactive cobalt wire (Co^{60}) has been suggested by Myers (171) as a substitute for radium The strong gamma radiation with a half-value thickness in lead of 0 41 inch makes the radioactive cobalt wire suitable for many therapeutic purposes Needles of a cobalt nickel alloy a few centimeters long can be made radioactive in the chain-reacting pile and have demonstrated uniform radioactivity. The ease of bending such a wire into any shape, convenience in handling since it is magnetic and can be manipulated with a strong electromagnet, and relative inexpensiveness, are its advantages over radium.

The application of the radioactive isotopes to therapy is still in its infancy and further trial with labeled elements and compounds will undoubtedly uncover many that will result in great diagnostic and therapeutic advances.

Dosimetry in Radioisotope Therapy

The rational use of radioisotopes in medicine, for either radiation therapy or tracer studies, requires the expression of tissue dose in familiar dosage units It is not now possible to do this as a general

spleen of mice, and *in vivo* fission of the isotope by slow neutron bombardment resulted in the release of large amounts of ionizing radiation in these tissues The biologic effect per unit of tissue ionization was greater with the fission recoils than with beta particles. However, the therapeutic implications of this are obviously limited.

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The ability to distribute radioactive colloids to various tissues by intravenous injection and its strict localization at the point of infiltration when injected into or around tissues has interesting therapeutic and diagnostic possibilities Further trial with these compounds is certainly indicated

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OTHER ELEMENTS

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solid or liquid. As a result, it is customary to base the estimate of radiation exposure upon a somewhat different effect, namely, the ionization created in air by the radiation in question. The unit of dosage on this basis is the roentgen, which is defined as that amount of x- or gamma radiation which produces 1 electrostatic unit of ionization in 1 cc. of air under standard conditions.

Since the effective atomic numbers of tissue and of air are approximately equal, it is reasonable to suppose that ionization in tissue is approximately proportional to ionization in air. Thus, the roentgen is a satisfactory unit for measuring exposure to all x- and gamma radiation. The actual realization of a measurement in roentgens is beset by difficulties (205,229).

When an attempt is made to apply the practices of x-ray dosimetry to the radioisotopes, a number of difficult problems arise The isotope may enter the body by injection, ingestion, or inhalation. It is taken up by various tissues at different rates, then eliminated at different rates from the various tissues and from the body. The result is so complex that it is impossible in most cases to make accurate dosage estimates. Only in certain special cases, where some broad assumptions are justified, is it possible to compute dosages In applying the formulas given below, it is essential to check the assumptions and limitations by which the formula was derived against the actual situation of the radioisotope in the body.

BETA RADIATION

The general aspects of the dosimetry of the beta particles have been discussed by several authors (50–52,57,153,156,157). The most complete and useful formulation is that by Marinelli and co-workers (156,157), and the discussion here will follow their formulation. In order to put beta dosimetry on an approximately comparable basis with x-ray dosimetry, an *equivalent roentgen* (e.r) is defined as "that amount of beta radiation which releases in one gram of air as much energy as one roentgen of gamma rays " For practical purposes this is the same unit as the *roentgen equivalent physical* ("rep") (50)

The range in tissue of beta particles is only a few millimeters For example, for I^{131} and P^{32}, the maximum beta range in tissue is about 2 and 8 mm , respectively, while the average range is about one-fourth the maximum. Thus, the energy given to a beta particle

rule, but under certain limited conditions a satisfactory expression of the tissue dose following administration of a radioisotope may be accomplished.

The radiations of primary importance in medicine at the present time are x-rays (electromagnetic radiation originating in the atomic electrons), gamma rays (electromagnetic radiation originating in an atomic nucleus), and beta particles (high-speed electrons ejected from an atomic nucleus). An attempt can be made to base the dosimetry of the gamma rays and beta particles upon the well-established principles of x-ray dosimetry. The dosimetry of the nuclear radiations which at present have a very limited use, namely, alpha particles (high speed helium nuclei), protons (hydrogen nuclei), neutrons (uncharged particles of proton mass), and deuterons (high speed neutron-proton pairs) will not be considered

The absorption in tissue of both x-ray and gamma ray photons (a photon is the unit of electromagnetic radiation) gives rise to scattered, secondary photons and also to secondary electrons Within the limit of the energy now available (say, up to 5 million volt x- or gamma rays), the secondary electrons are created by either the photoelectric or Compton recoil processes These secondary electrons, created with relatively high velocities, are in turn slowed down by collisions with the atomic electrons in the tissue Some of these collisions are sufficiently close to eject the atomic electron altogether, leaving a positively charged, or ionized atom Other collisions, less violent, leave the atom intact, still neutral, but excited It is believed that the disruption of chemical bonds arising from the ionization (but not the excitation) is responsible for the biologic action of penetrating radiation

Beta particles (primary electrons) are slowed down by exactly the same collision processes as are the secondary electrons from x- and gamma rays Thus, all penetrating radiations produce their effect by creating ionization throughout the tissue reached by primary or secondary radiation An excellent review of current views on the mechanism of the biologic action of radiation has recently been given by Lea (140)

From the preceding discussion, it might seem that the ideal unit for dosimetry would be one based upon ionization in tissue. There is, however, no satisfactory method of measuring ionization in a

It will be found to be characterized by an "effective half-life," represented here by the symbol T_{eff}. Moreover, these three half-lives are related by the following equation (assuming that the biologic half-life *represents an exponential process*):

$$\frac{1}{T_{eff}} = \frac{1}{T} + \frac{1}{T_b}, \quad \text{or} \quad \frac{T_{eff}}{T} = \frac{T_b}{T + T_b} \tag{3}$$

Equation (1) is derived assuming that the biologic half-life is very much greater than the radioactive half-life. In general, this is not true, and a more general equation for the beta dose is:

$$D_\beta = K_\beta \left(\frac{T_{eff}}{T}\right) C \tag{4}$$

in equivalent roentgens, where D_β is the radiation dose given to tissue by C microcuries per gram of a given isotope with a radioactive half-life of T days, uniformly distributed throughout a region of tissue larger than the range of the beta particles, and which is disappearing from the tissue with an actual half-life of T_{eff}. D_β is the dose within the region of uniform distribution, but not near the surface Moreover, D_β is the total dose, from the time at which the concentration is C until the isotope has completely disappeared, assuming that T_{eff} is constant until complete disappearance.

The beta dosage factor K_β and the radioactive half-life T are physical constants, which have been tabulated for radioisotopes of current medical interest The effective half-life T_{eff} and the concentration C are factors which must be estimated independently for each patient, in applying equation (4) Under some circumstances (e g., I^{131} and thyroid uptake) the effective half-life can be measured directly by means of a Geiger counter. Under other circumstances (e.g., P^{32} in bone marrow), the effective half-life must be estimated from information on the rate at which the material in question will be metabolized, that is, from an estimate of the biologic half-life The way in which the factor (T_{eff}/T) of equation (4) depends upon the ratio (T_b/T) is shown in the following tabulation

(T_b/T) =	20	10	3	1	1/3	1/10	1/20
(T_{eff}/T) =	0 95	0 91	0 75	0 50	0.25	0 09	0 05

It is seen that if the biologic half-life T_b is greater than ten times the radioactive half-life T, we can assume that the effective half-life equals the radioactive half-life with an error of not over 10 per cent.

by a radioactive atom is delivered to the tissue within a few millimeters of the atom. As a result, the rate at which energy is delivered to tissue by a uniform distribution of radioisotope is just the average energy of a beta particle times the number of particles per unit time. From this, it is easily shown that the dosage given to tissue can be expressed by·

$$D_\beta = K_\beta C \qquad (1)$$

in equivalent roentgens, where K_β is a constant for a given isotope, C is the concentration of the radioisotope in tissue in microcuries per gram (μc/Gm.) and where it is assumed that the radioisotope remains in the tissue until completely decayed

The beta dosage constant, K_β, is given by:

$$K_\beta = 88\,\overline{E}_\beta T \qquad (2)$$

in equivalent roentgens per microcurie destroyed per gram, where $\overline{E}_\beta$ is the average energy of the beta particles in million electron volts (m e v) and T is the radioactive half-life of the isotope in days Thus K_β is a constant for each isotope, and need be computed only once. Values of K_β for many of the isotopes of medical interest are given in Table II. The units given for K_β in equation (2) indicate that K_β gives directly the tissue dose in equivalent roentgens of 1 μc./Gm (or 1 mc /Kg) allowed to remain in the tissue until completely disintegrated

The rate of radioactive disintegration is expressed by a *half-life,* designated here by the symbol T, which has the following significance half the atoms disintegrate in T days, then half the remaining atoms disintegrate in the next T days, etc. T is a constant for a given isotope, and cannot be influenced in any way. Thus, when a radioisotope is distributed in a mass of tissue, the number of atoms present will decrease both as a result of radioactive decay, and as a result of metabolic elemination (if one assumes that uptake has been completed) As long as the metabolic process can be considered reasonably uniform, the biologic rate of decrease will be constant and can be at least approximately characterized by a "biologic half-life," represented here by the symbol T_b If the actual total rate of decrease of the radioisotope in tissue is observed (as, for example, with a Geiger counter), it will be found that this actual rate is greater than that due to either radioactive or biologic half-life alone.

It will be found to be characterized by an "effective half-life," represented here by the symbol T_{eff}. Moreover, these three half-lives are related by the following equation (assuming that the biologic half-life represents an exponential process):

$$\frac{1}{T_{eff}} = \frac{1}{T} + \frac{1}{T_b}, \quad \text{or} \quad \frac{T_{eff}}{T} = \frac{T_b}{T + T_b} \tag{3}$$

Equation (1) is derived assuming that the biologic half-life is very much greater than the radioactive half-life. In general, this is not true, and a more general equation for the beta dose is:

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in equivalent roentgens, where D_β is the radiation dose given to tissue by C microcuries per gram of a given isotope with a radioactive half-life of T days, uniformly distributed throughout a region of tissue larger than the range of the beta particles, and which is disappearing from the tissue with an actual half-life of T_{eff}. D_β is the dose within the region of uniform distribution, but not near the surface. Moreover, D_β is the total dose, from the time at which the concentration is C until the isotope has completely disappeared, assuming that T_{eff} is constant until complete disappearance

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(T_b/T) =	20	10	3	1	1/3	1/10	1/20
(T_{eff}/T) =	0 95	0 91	0 75	0.50	0 25	0 09	0 05

It is seen that if the biologic half-life T_b is greater than ten times the radioactive half-life T, we can assume that the effective half-life equals the radioactive half-life with an error of not over 10 per cent

But, if the biologic half-life is less than ten times the radioactive half-life, an error of more than 10 per cent in dosage estimate will result if we assume $T_{eff} = T$. In the event that no reliable estimate of the effective half-life can be made, and equation (4) is used with $T_{eff} = T$—which is equivalent to using equation (1)—the resulting estimate of D_β is an upper limit to the actual beta dose, since the factor (T_{eff}/T) is always less than unity.

Getting a reliable estimate of the concentration C, in microcuries per gram, is never easy. Under the most favorable circumstances, when it is valid to assume uniform distribution of the isotope in the tissue, it is still necessary to know: *(1)* the true dose administered, in microcuries, *(2)* the fraction taken up by the tissue; and *(3)* the weight of the tissue. Each of these three is a very difficult datum to secure. Under unfavorable circumstances, where the distribution of the isotope is not uniform, use of equation (4) will underestimate the dose in some regions and overestimate it in others.

If the tissue which has taken up the radioisotope is smaller in its linear dimensions than the range of the betas, the use of equation (4) will again give an upper limit to the tissue dose, provided its other conditions are satisfied. The only nonequilibrium distribution for which some dosage estimate can be made, is the following: when a radioisotope is uniformly distributed throughout a tissue considerably larger than the range of the betas, the tissue dose at the surface of the tissue will be one-half the value given by equation (4). The tissue carrying the radioisotope must be surrounded by other tissue at least as thick as the range of the beta particles.

GAMMA RADIATION

Gamma rays of energy similar to that of radium are absorbed at a rate of about 3 per cent per centimeter in tissue. The result of this relatively small absorption is that the energy of the gamma rays is distributed throughout a large volume around each point source of gamma radiation. This fact is of course a basic consideration in radium therapy, and there exists a very extensive literature concerning the calculation of tissue dose around various configurations of radium sources (74,162). The dosimetry of the artificially radioactive gamma ray emitters does not differ in principle from that of radium. Certain additional factors must be taken into ac-

count, namely, the radioactive half-life, the biologic half-life, and the distribution of the isotope throughout the tissues of the body. When these are formulated mathematically, it is shown that the gamma dose can be expressed by three equations analogous to equations (1), (2), and (4):

$$D_\gamma = K_\gamma C g \tag{5}$$

roentgens, where

$$K_\gamma = 24 \times 1.44 \times 10^{-3} I_\gamma T \tag{6}$$

roentgens at 1 cm /μc destroyed. Then

$$D_\gamma = K_\gamma \frac{T_{eff}}{T} C g \tag{7}$$

roentgens Eq (7) is the more general, since it includes effective half-life T_{eff} and the radioactive half-life T while equation (5) holds only when the biologic half-life is so long that $(T_{eff}/T) = 1$ C is the concentration in microcuries per gram. I_γ is defined as the roentgens per millicurie-hour at 1 cm. in air for the isotope in question. (For radium or radon filtered with 0 5 mm. platinum and in equilibrium with its disintegration products, $I_\gamma = 8\ 4$) Factor g of these equations takes into account the geometric configuration of the tissue between and around the source of radiation and the point where the dose is being determined. The intensity of the radiation from a very small source falls off with the square of the distance, and also is absorbed at the rate of about 3 per cent per centimeter of tissue The full expression for g is a mathematical expression, an integral, for these two effects

If the gamma emitter is distributed in a sphere of tissue of radius R, and if we are determining the dose at the center, then $g = 4\pi R$, provided R is not greater than about 10 cm For a point source of gamma radiation, $g = 1/d^2$ (approximately) if d is the distance to the source, and if we neglect absorption in the tissue For more complicated cases, the computation of g becomes lengthy and involved

Values of K_γ, I_γ, and T are given for a number of isotopes in Table II. A complete tabulation for isotopes of medical interest is given by Marinelli, *et al* (157) The other factors of equations (4) and (7) must be determined for each application Thus, in addition to the difficulties of determining the tissue concentration C, and the effective half-life T_{eff}, both of which are inherent in beta dosimetry,

gamma dosimetry has the usually formidable requirement of computing a geometric factor g which is dependent upon the size and shape of the tissues and upon the distribution of the radioisotope.

In the foregoing discussion, we have tacitly assumed, for the sake of simplicity, that the radioisotope is taken up instantly by the tissue in question. In those cases where the rate of uptake is appreciably slow, it can be taken into account, if known, by a suitable mathematical extension of the methods outlined here. Likewise, we have omitted mention of the isotopes which decay by electron capture, positron emission, etc., as being too specialized for the present general treatment.

EXAMPLES OF DOSAGE COMPUTATIONS

Radioactive Phosphorus P^{32}

This isotope, which has been used in medicine perhaps more widely than any other, is in some respects a favorable one for the computation of tissue dose. It decays by beta emission only, without gamma radiation. The relevant physical information is as follows:

radioactive half-life, $T_r = 14.3$ days
maximum range in tissue, approx 8 mm
beta dosage factor, $K_\beta = 885$ e r/μc destroyed per gram

Jones and co-workers (121) used P^{32} in the form of colloidal, anhydrous chromic phosphate Following intravenous administration in mice and a dog, they found that about 90 per cent of the P^{32} went to the liver, a few per cent each to the spleen and to the lung, and negligible amounts to the remaining tissues. They also found a negligible rate of excretion over a period of many months If, in the absence of other information, one assumes that these results apply to humans, one can now calculate the dose to the liver of a patient administered, say, 10 mc of P^{32} in the form of chromic phosphate Assuming that the weight of the liver is 1.7 Kg, the concentration of P^{32} in the liver is then

$$C = \frac{10{,}000 \times 0.90}{1{,}700} = 5.3\ \mu c/\text{Gm.}$$

Then, from equation (1).

$$D = 885 \times 5.3 = 4{,}700 \text{ equivalent roentgens}$$

To obtain this number we made the explicit assumptions of a 90 per cent uptake in the liver, a 1.7 Kg liver, and a biologic half-life

that is long compared to 14.3 days. We also made the implicit assumptions that the P^{32} is uniformly distributed throughout the liver, and that the dimensions of the liver are large compared to the range of the beta particles.

If P^{32} is administered in soluble form, the distribution is rather different. The highest uptake per gram is in the bone marrow, followed by spleen, liver, and kidney, and the excretion is appreciable. The dosage considerations in this case are examined in detail by Marinelli *et al.*

From the value of the beta dosage factor, K_β, given above, it follows that 1 μc of P^{32} destroyed per kilogram of tissue equals 0 9 equivalent roentgen It also follows that 1 mc. of P^{32} administered to a 70 Kg. man gives 12 6 equivalent roentgens of whole body radiation, assuming uniform distribution in the tissues and no excretion

Radioactive Iodine, I^{131}

This isotope emits both beta and gamma radiation. The physical information needed for dosage computation is as follows:

radioactive half life, $T = 8\ 0$ days
maximum range of betas in tissue, approx 2 mm
beta dosage factor, $K_\beta = 144$ e r /μc destroyed per gram
gamma dosage factor, $K_\gamma = 0\ 735$ r at 1 cm /μc. destroyed

Let us assume, for a sample calculation, a 24 Gm thyroid, consisting of 2 equal spheres about 1 inch apart Then each sphere will have a radius of about 1 4 cm. Let us further suppose that the effective half-life is measured on the patient and found to be 6 5 days (157), and that about half the orally administered iodine goes to the thyroid, and about half is excreted, with a small fraction distributed throughout the rest of the body.

The concentration of the radioisotope in thyroid tissue, for a 10 mc dose, is then

$$C = \frac{10{,}000 \times 0\ 50}{24} = 210\ \mu c/\mathrm{Gm}$$

So then, the beta dose is given by equation (4) as

$$D = 144 \times (6\ 5/8\ 0) \times 210 = 25{,}000 \text{ equivalent roentgens}$$

To compute the gamma dose, we need the geometrical factor

$$g = 4\pi R = 4\pi \times 1\ 4 = 17.6$$

TABLE II. Physical Properties of the Radioisotopes Used in Medicine

COLUMN

1. Atomic number
2. Chemical symbol of the element and mass number of the isotope
3. Types of radiation emitted The symbols have the following meaning β^- = negative beta particle, β^+ = positive beta particle (positron), γ = gamma rays, K = x-rays following K-electron capture, e^- = electrons from internally converted gamma rays, and α = alpha particles Beta decay known to be without gamma radiation is specifically indicated as "no γ" Positron decay known to be without K capture is marked "no K" Percentage of decay of each type is given in parentheses, if known
4. The radioactive half life in minutes, hours, days, or years
5. The fraction of the isotope decaying during 24 hours, $fd = (1 - e^{-0\,693/T})$, where T is the radioactive half life expressed in days
6. The maximum energy in m e v of the beta particle spectrum, or the alpha particle line. More than one number means a compound spectrum
7. The average energy in m e v of the beta particle spectrum, $\overline{E}_\beta$, where known The asterisk (*) indicates that part or all of the energy given is actually due to the soft x rays which follow K-capture For elements of medium and light atomic weight, these x rays are absorbed so rapidly in tissue that they may be treated like beta particles for the purpose of computing dosage

COLUMN

8. The beta dosage factor, $K_\beta = 88\ E_\beta T$ It is the dose due to complete disintegration of 1 μc per gram of tissue of a beta-emitting radioisotope, uniformly distributed throughout a volume of tissue of dimensions larger than the range of the beta particles The asterisk (*) has the same meaning as in column 7.
9. The maximum range, in millimeters of water, of the beta or alpha particles In the case of a compound spectrum, the largest value is given. An asterisk (*) indicates that the value given is the distance for 95 per cent absorption (in water) of the x rays which follow K-capture. This value is given only when it is larger than the maximum range of the beta particles (if any), and when the corresponding K_β includes a contribution from these x rays The range in tissue will be essentially the same as the range in water
10. The energies in m e v of the gamma ray photons The 0 51 m e v. annihilation gammas which accompany positron decay are stated specifically in parentheses.
11. The gamma dose-rate, I_γ, which is the number of roentgens per hour at 1 cm. in air from a point source of 1 mc (I_γ = 8 4 for radium or radon in equilibrium with its decay products, and filtered with 0.5 mm of platinum)
12. The gamma dosage factor, $K_\gamma = 1\ 14 \times 24 \times 10^{-3}\ T\ I_\gamma$ It is the dose in air at 1 cm from a point source, for complete disintegration of 1 μc
13. A plus sign indicates that the isotope is produced in a nuclear pile, and is available from the U S. Atomic Energy Commission, Isotope Branch, Oak Ridge, Tenn

Z	Isotope	Types of radiation	Radioactive half life	Frac disintegrating in 1 day	Particles and x radiation: Max energy, m e v	Av energy, m e v	Dosage factor,[a] K_β	Max range in H_2O, mm.	Gamma radiation: Energy, m e v	Dose rate,[b] I_γ	Dosage factor,[c] K_γ	Pile produced
1	2	3	4	5	6	7	8	9	10	11	12	13
1	H^3	β^-, no γ	11 y	0 000	0 013			0 003				+
6	C^{11}	β^+, no γ	20 m	1 00	1 0	0 380	0 47	4	(0 51)	6 2	0 003	
	C^{14}	β^-, no γ	5100 y	0 000	0 15	0 05	8×10^6	0 3				+
7	N^{13}	β^+, no γ	10 0 m	1 00	0 9, 1 2	0 475	0 29	6	(0 51)	6 2	0 0015	
9	F^{18}	β^+, γ	112 m	1 00	0 6(80%), 0 9(20%)				(0 51), 1 4			

1	2	3	4	5	6	7	8	9	10	11	12	13
11	Na^{22}	β^+, no K, γ	3.0 y	0.001	0.6	0.225	2.2×10^4	2	(0.51), 1.3	13.2	500	
	Na^{24}	β^-, γ	14.8 h	0.68	1.4	0.510	29	6	1.4, 2.8	19.1	0.40	+
15	P^{32}	β^-, no γ	14.3 d	0.047	1.7	0.695	885	8				+
16	S^{35}	β^-	87.1 d	0.008	0.17	0.055	420	0.3				+
17	Cl^{38}	β^-, γ	38 m	1.00	1.2(36%), 2.7(11%), 5.2(53%)	1.398	3.2	26	1.6(43%), 2.1(57%)	7.6	0.0068	
18	A^{41}	β^-, γ	110 m	1.00	1.2			5	1.3			
19	K^{42}	β^-, γ	12.4 h	0.74	2.0(25%), 3.6(75%)	1.395	63	18	1.5, 2.1	1.93	0.035	+
20	Ca^{45}	β^-, no γ	152 d	0.005	0.25	0.10	1580	0.6				+
25	Mn^{52}	β^+ (35%) K (65%), γ	5.8 d	0.11	0.7	0.085	48	3	(0.51), 0.7, 0.9, 1.5	19.5	4.4	
	Mn^{54}	K, γ	310 d	0.002		0.0051*	147*	1.0*	0.84	4.9	52	
	Mn^{56}	β^-, γ	2.59 h	1.00	0.75(20%), 1.0(30%), 2.8(50%)	0.890	8.5	14	0.8(~100%), 1.8(30%), 2.1(20%)	9.4	0.035	
26	Fe^{55}	K, no β^+ no γ	~4 y	0.000		0.0059*	780*	1.2*				+
	Fe^{59}	β^-, γ	45 d	0.015	0.26 0.46	0.120	496	1.4	1.1, 1.3	6.55	10.7	+
27	Co^{56}	β^+, γ, K	72 d	0.010	1.4	0.655	4900	6	(0.51), 0.85, 1.3, 1.7, 2.0, 2.6, 3.2	18.0	37.2	
	Co^{57}	K, γ, e^-, β^+	270 d	0.003	β^+ 0.26			0.6	0.12, 0.20, (0.51)			
	Co^{58}	β^+ (15%), K (85%), γ	72 d	0.010	0.47	0.035*	20*	1.6*	(0.51), 0.8	5.7	12.8	
	Co^{60}	β^-, γ	5.3 y	0.000	0.31	0.099	1.7×10^4	0.8	1.2, 1.3	13.5	900	+

Table continued

TABLE II Physical Properties of the Radioisotopes Used in Medicine

COLUMN

1 Atomic number
2 Chemical symbol of the element and mass number of the isotope
3. Types of radiation emitted The symbols have the following meaning β^- = negative beta particle, β^+ = positive beta particle (positron), γ = gamma rays, K = x rays following K-electron capture, e^- = electrons from internally converted gamma rays, and α = alpha particles Beta decay *known to be without* gamma radiation is specifically indicated as "no γ" Positron decay *known* to be without K capture is marked "no K" Percentage of decay of each type is given in parentheses, if known
4 The radioactive half-life in minutes, hours, days, or years
5 The fraction *of the isotope decaying* during 24 hours, $fd = (1 - e^{-0.693/T})$, where *T* is the radioactive half-life expressed in days
6 The maximum energy in m e v of the beta particle spectrum, or the alpha particle line More than one number means a compound spectrum
7 The average energy in m e v of the *beta particle spectrum*, $\overline{E}_\beta$, where known The asterisk (*) indicates that part or all of the energy given is actually due to the soft x rays which follow K capture For elements of medium and light atomic weight, these x rays are absorbed so rapidly in tissue that they may be treated like beta particles for the purpose of computing *dosage*

COLUMN

8 The beta dosage factor, $K_\beta = 88\ E_\beta T$ *It is the dose due* to complete disintegration of 1 μc per gram of tissue of a beta-emitting radioisotope, uniformly distributed throughout a volume of tissue of dimensions larger than the range of the beta particles The asterisk (*) has the same meaning as in column 7.
9 The maximum range, in millimeters of water, of the beta or alpha particles, In *the case* of a compound spectrum, the largest value is given An asterisk (*) indicates that *the value given is the distance for 95* per cent absorption (in water) of the x rays which follow K capture. This value is given only when it is larger than the maximum range of the beta particles (if any), and when the corresponding K_β includes a contribution *from these x rays* The range in tissue will be essentially the same as the range in water.
10 The energies in m e v of the gamma ray photons The 0 51 m e v. annihilation gammas which accompany positron decay are stated specifically in parentheses
11 The gamma dose rate, I_γ, which is the number of roentgens per hour at 1 cm, in air from *a point source* of 1 mc (I_γ = 8 4 for radium or radon in equilibrium *with its* decay products, and filtered with 0 5 mm of platinum)
12 The gamma dosage factor, $K_\gamma = 1\ 44 \times 24 \times 10^{-3}\ T\ I_\gamma$ It is the dose in air at 1 cm from a point source, for complete disintegration of 1 μc
13 A plus sign indicates that the isotope is produced in a *nuclear pile, and* is available from the U S. Atomic Energy *Commission*, *Isotope* Branch, Oak Ridge, Tenn

Z	Isotope	Types of radiation	Radioactive half-life	Frac disintegrating in 1 day	Particles and x radiation: Max energy, m e v	Av energy, m e v.	Dosage factor,[a] K_β	Max range in H_2O, mm.	Gamma radiation: Energy, m e v	Dose rate,[b] I_γ	Dosage factor,[a] K_γ	Pile produced
1	2	3	4	5	6	7	8	9	10	11	12	13
1	H^3	β^-, no γ	11 y	0 000	*0 013*			0 003				
6	C^{11}	β^+, no γ	20 m	1 00	1 0	0 380	0 47	4	(0 51)	6.2	0 003	
	C^{14}	β^-, no γ	5100 y	0 000	0 15	0 05	8 × 10°	0 3				+
7	N^{13}	β^+, no γ	10 0 m	1 00	0 9, 1 2	0 475	0 29	6	*(0 51)*	*6 2*	*0 0015*	
9	F^{18}	β^+, γ	112 m	1 00	0 6(80%), 0 9(20%)				(0 51), 1 4			+

1	2	3	4	5	6	7	8	9	10	11	12	13
40	Zr^{89}	β^+, no γ	80 h	0 19	1 0			4	(0 51)			+
	Zr^{95}	β^-, e^-, γ	65 d	0 011	0 4(96%), 1 0(4%)			4	0 23(93%), 0 7 (93%), 0 9 (7%)			+
41	Cb^{95}	β^-, γ	36 d	0 019	0 15			0 3	0 8			+
51	Sb^{124}	β , γ	60 d	0 012	0 6, 1 0, 1 6, 2 4	0 66	3480	11	0 12, 0 6, 1 7	7 9	16 4	+
53	I^{130}	β^-, γ	12 6 h	0 73	0 6, 1 0	0 270	12 4	4	0 12, 0 54, 0 67, 0 74	13 0	0 237	
	I^{131}	β^-, γ, e^-	8 0 d	0 083	0 6	0.205	144	2	0 37, 0 080	2 65	0 735	+
79	Au^{198}	β^-, γ, e^- (5%)	2 7 d	0 23	0 6(15%), 1 0(85%)	0 32	76	3	0 16(15%), 0 21(15%), 0 41(100%)	2 4	0 22	+
85	At^{211}	α (60%) K (40%)	7 5 h	0 89	α 5 9			0 06				

The data in columns 3, 4, 6, and 10 is from Seaborg and Perlman (197) Where these authors quoted several values, what seemed to be the best value was chosen in some cases, and an average was used in other cases The data in columns 7, 8, 11, and 12, is from Marinelli, Quimby, and Hine (157), without change Dosage factors computed from the data of Seaborg and Perlman will in some cases differ those given by Marinelli *et al*, since the data of the former authors is the more recent The data of columns 5 and 9 follow the practice of Marinelli *et al*, but have been brought into line with the adopted values in columns 4 and 6, and the "range" of the x rays of K capture has been added

The "safe tracer concentration," given by Marinelli *et al*, may be easily computed from the data of columns 5 and 8 as follows $S_\beta = 0\,1/K_\beta/_d$ is the concentration, expressed in $\mu c/Kg$, of a beta emitting radioisotope which will deliver to tissue a dose of 0 1 equivalent roentgens during the first 24 hours of exposure For half lives greater than 10 days, a simple formula is $S_\beta = 1\,6 \times 10^{-3}/\overline{E}_\beta$.

[a] e r/μc destroyed per gram   [b] r at 1 cm per millicurie hour   [c] r at 1 cm per microcurie destroyed

TABLE II (*continued*). Physical Properties of the Radioisotopes Used in Medicine

Z	Isotope	Types of radiation	Radioactive half-life	Fraction disintegrating in 1 day	Particles and x radiation: Max energy, m e v	Particles and x radiation: Av energy, m e v.	Particles and x radiation: Dosage factor,[a] K_β	Particles and x radiation: Max range in H_2O, mm	Gamma radiation: Energy, m e v.	Gamma radiation: Dose rate,[b] I_γ	Gamma radiation: Dosage factor,[c] K_γ	Pile produced
1	2	3	4	5	6	7	8	9	10	11	12	13
29	Cu^{64}	β^- (65%), β^+ (32%), K, γ (3%)	12 8 h	0 73	β^- 0 58, β^+ 0 66	0 120	5 6	2	(0 51), 1 3(3%)	1 2	0 022	+
30	Zn^{63}	β^+, γ, K (?)	38 m	1 00	2 3	0 965	2 3	11	(0 51), 1 0, 1 9, 2 6	6 9	0 0065	
	Zn^{65}	β^+ (1 3%), K (98 7%), γ, e^-	250 d	0 003	β^+ 0 4	0 01*	180*	4*	0 45, 0 65, 1 1	3 0	26	+
33	As^{74}	β^-, β^+, γ	18 d	0 038	β^- 1 3, β^+ 0 9			5	(0 51), 0 58			
	As^{76}	β^-, β^+, K, γ	26 8 h	0 46	β^- 1 3(15%), 2 5(25%), 3 0(60%), β^+ 0 7, 2 6	1 170	115	16	(0 51), 0 57(∼70%), 1.2(∼30%)	2 2	0 083	+
35	Br^{82}	β, γ	34 h	0 39	0 46	0 150	20	1 4	0 55, 0 79, 1 4	15 1	0 79	+
36	$Kr^{79,81}$	β^+, γ	34 h	0 39	∼0 6(70%), ∼0 9(30%)			3	0 2, (0 51)			
37	Rb^{86}	β^-, γ	19 5 d	0 035	1 6			7				+
38	Sr^{85}	K, γ	65 d	0 011				15*	0 8			
	Sr^{89}	β^-, no γ	55 d	0 013	1 5	0 57	2760	7				+
	Sr^{90}	β^-, no γ	∼30 y	0 000	0 6	0 22	17×10^4	2				+
39	Y^{88}	K, γ	105 d	0 007		0 005*	46*	18*	0 9, 1 9, 2 8	17	62	-
	Y^{90}	β, no γ	62 h	0 24	2 2	0 90	200	10				+
	Y^{91}	β^-, no γ	57 d	0 012	1 5			7				+

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at the center of one spherical lobe. Then the gamma dose is given by equation (7):

$$D = 0.735 \times (6.5/8.0) \times 210 \times 17.6 = 2200 \text{ roentgens}$$

To compute the dose which one lobe gives to the other, we note that the distance between centers is $d = 2.5 + 2.8 = 5.3$ cm. Therefore $g = 1/5.3^2 = 0.036$, and the dose is $2{,}200 \times (0.036/17.6) = 4$ roentgens, which is a negligible fraction of the other dosages. Thus, the total dose at the center of each lobe is 27,000 roentgens.

To secure this number, it was necessary to make explicit assumptions concerning the weight of the thyroid in grams, the shape of the thyroid, the effective half-life, and the fraction of the administered dose taken up by the thyroid. The implicit assumptions were rapid uptake in the gland, uniform distribution in the gland, and simple additivity of equivalent roentgens and gamma roentgens.

It should be clear from this example that the dosage computation for the relatively favorable case of the thyroid is at best only approximate. For most applications of radioisotopes to internal medicine, it is not possible to make even these crude estimates at the present time.

Note added in proof: The computations of dosage by beta radiation given here were based upon an arbitrary definition of the equivalent roentgen (or rep), of 83 ergs per gram absorbed energy. Recently, the Subcommittee on the Handling of Radioactive Isotopes, of the National Committee on Radiation Protection, adopted the definition 93 ergs per gram absorbed energy for the rep (171a). This brings the definition more nearly into line with the energy actually absorbed by tissue from a roentgen of x-rays. If this new numerical value is used, the values of K_β, as computed from equation (2), or as listed in column 8 of Table II, would be *decreased* by 10 per cent. Thus the computed beta doses in the examples would be 10 per cent smaller. This new value will probably be adopted in future usage.

The radioactive iodine and phosphorus used in the investigations reported here from our laboratory, were supplied on allocation from the Isotopes Division, United States Atomic Energy Commission.

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Brucellosis*

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Introduction

During the more than twenty-five years which have elapsed since the initial demonstration in this country of human infection due to Brucella, the disease has come to be recognized as the most common illness transmitted to humans from animals The number of cases reported yearly has steadily increased, to some extent due to the fact that both physicians and patients are more aware of this disease. But there is also good reason for believing that more human infections have actually occurred. During the World War II, several factors contributed to the spread of brucellosis. Of major importance was the deterioration of control of the disease in livestock. A shortage of veterinary and farm personnel developed just when animal production rose to meet the enormous wartime demand for dairy products and meats As a consequence, susceptible and infected animals were moved about and exchanged with inadequate veterinary supervision. The effect of these circumstances on the animal reservoir is clearly demonstrated in Table I.

The impression that the incidence of human brucellosis is increasing is difficult to substantiate with satisfactory data The precise methods which are necessary and yet often inadequate for exact diagnosis in the individual case obviously cannot be applied to surveys of large population groups For many years this problem has been carefully studied in Iowa, and the results have indicated that in this state there has been a marked increase in the morbidity rate. According to Jordan (39), brucellosis has increased from 5 31 per 100,000 during the 5 year period of 1935–1939 to 13 per 100,000 in the period 1940–1944.

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TABLE I

Comparative Incidence of Infected Cattle in Midwestern States at the Beginning and Close of World War II (Based on Agglutination Tests)*

State	Per Cent Reactors in	
	1941	1946
Illinois	3.7	5.9
Indiana	1 6	9.3
Iowa	5 3	9.5
Kansas	3 5	8 5
Michigan	3 2	1.9
Minnesota	2 7	8.2
Missouri	2 9	7.5
Nebraska	3 8	7.6
North Dakota	3 8	7 6
Ohio	3 8	6 0
South Dakota	4 2	8 8
Wisconsin	2 6	5 8

* Compiled from data submitted to Conference on Brucellosis Control sponsored by the U. S Department of Agriculture, Bureau of Animal Industry, at Stockyards Inn, Chicago, June 1948.

In order to estimate the importance of recent progress in the study of human brucellosis, it is necessary to bear in mind some outstanding contributions made in the past. These may be listed as follows.

Bruce	1887	Isolated the causative agent from human spleens and reproduced the infection in monkeys.
Bang	1895	Isolated Brucella abortus from fetuses of aborting cattle
Hughes	1897	Described the natural history of undulant fever as he observed it in a large series of cases
Wright and Semple	1897	Demonstrated agglutinins in the serum of infected patients
Mediterranean Fever Commission	1904–7	Discovered that goats were the source of the infection in Malta Established basic epidemiologic principles
Craig	1905	Reported first human case originating in this country
Schroeder and Cotton; Smith and Fabyan	1911	Transmitted Br. abortus from cows milk to guinea pigs and accurately described the lesions.
Larson and Sedgwick	1913	Found complement fixing antibodies for Br abortus in the serum of children drinking raw milk

Traum	1914	Isolated Br. suis from fetuses expelled from sows
Evans	1918	Demonstrated close relationship between Br. melitensis and Br. abortus
Fleischner and Meyer	1919	Elicited dermal hypersensitivity in guinea pigs infected with Br. abortus
Keefer	1924	Isolated Br. suis from first human case in this country.
Morales Otero	1929	Experimentally inoculated and fed human volunteers
Huddleson	1931	Biochemical differentiation of the species of Brucella.
Meyer	1943	Observed intracellular parasitism in tissues of a fatal case

By virtue of the foregoing observations, a basic understanding has evolved of the epidemiology, immune reactions, animal pathology, clinical syndromes, and diagnostic principles. Knowledge still lacking, however, is a satisfactory description of the pathogenesis of the disease in humans and a successful specific therapy. Significant developments have occurred recently in both categories, and will be dealt with in detail. In addition, contributions of importance in each of the other fields of study will be considered

Epidemiology

The elimination of human brucellosis is dependent upon the eradication of the disease in animals. With this in mind, any enthusiasm over recent developments offering relief to the individual patient must be tempered by a realization that the number of infected animals has been increasing in this country (Table I) A study of the complexities of animal transmission to other animals, and to man, is discouraging Not only livestock, but other animals have been shown to harbor Brucella. Thus, it was found not long ago that equine brucellosis may be a source of human infection Carpenter and Boak (8) demonstrated agglutinins in the serums of 27 per cent of 347 horses. Some of these animals were asymptomatic, some became sterile, and in a few a disease known as fistulous withers developed. Significantly, 2 serious cases of brucellosis developed in children who had had contact with 1 horse having a purulent exudate from which Br abortus was isolated Other animals from which Brucella have been isolated are sheep, mules, deer, buffaloes, poultry, and dogs (34)

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Iowa	5.3	9.5
Kansas	3 5	8 5
Michigan	3 2	1 9
Minnesota	2.7	8.2
Missouri	2.9	7.5
Nebraska	3 8	7.6
North Dakota	3.8	7.6
Ohio	3 8	6 0
South Dakota	4.2	8 8
Wisconsin	2.6	5.8

* Compiled from data submitted to Conference on Brucellosis Control sponsored by the U S Department of Agriculture, Bureau of Animal Industry, at Stockyards Inn, Chicago, June 1948.

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University of Minnesota, several young hogs received in 11 months 15 inoculations of massive doses of a strain of Br suis highly virulent for the patient from whom it was recovered and lethal for guinea pigs in small numbers During the period of inoculation, the experimental subjects kept pace with the controls in every respect, including weight gain and apparent well-being Furthermore, a meticulous postmortem search for pathologic changes disclosed no abnormalities in the inoculated animals, despite the fact that Br suis was isolated from the liver, spleen, and other tissues Although high agglutinin titers were observed in the latter group of hogs, this is often not the case in many instances of porcine infections (36) Not infrequently, agglutination tests in infected animals are negative, or at least of low titers of a degree often seen in herds free of brucellosis This being the case, distinguishing diseased from normal hogs presents a most difficult problem

Of the three species causing brucellosis, Br abortus is generally regarded as the least invasive and virulent Nevertheless, it is the basis for widespread human illness, usually in sporadic form In Minnesota, Br abortus is by far the commonest cause of human brucellosis (Tables II, III), causing over 80 per cent of the cases,

TABLE II

Species of Brucella Isolated from the Blood of Patients by Laboratories of the Minnesota Department of Health from January 1, 1945, to July 1, 1948*

Brucella species	No of cases	Per cent of cases
Br abortus	268	85 9
Br suis	21	6 7
Br melitensis	23	7 4
Total	312	

* Courtesy of Dr Paul Kabler

and including a few which have terminated fatally In about one-fourth of the cases at the University of Minnesota Hospitals, and possibly in more, the portal of entry for this organism has been definitely established as the oropharynx (Table IV). This leaves no doubt regarding the importance of raw milk as a mode of transmission of abortus disease, regardless of some evidence to the contrary

New and disturbing complications have appeared in the problem of brucellosis control in livestock. Intended by history and nomenclature to belong solely to the hog, Br suis has invaded the cow. Similarly, Br. melitensis has infected the hog, and rarely, the cow In each instance, there has been ultimate transmission to human beings. These facts imply greater possibilities for dissemination of the more virulent and invasive strains of Brucella in this country Whereas Br. suis infections were formerly expected to be found among only those who experienced immediate contact with hogs, they must now be anticipated in large numbers of milk consumers who deal with dairies possessing herds infected with that organism As early as 1934, Beattie and Rice (1) described a milk-borne epidemic of 30 cases due to Br. suis, and since then several outbreaks having such an origin have been reported (4, 20, 39). It is usually found that these dairy cattle share their premises with infected hogs

Human infections due to Br. melitensis, which had been known to occur in the goat-raising areas of Mexico and the Southwest, were recognized in Iowa in 1943 (41) In the next 3 years, 40 cases of culturally proved melitensis infections were detected in that state Studies made in 20 of these patients disclosed that 12 had had direct contact only with hogs Final evidence for incriminating hogs was provided when the melitensis strain was isolated from the tissues of these animals in 1946 (10)

With alarming rapidity, melitensis infections have apparently spread to the neighboring state of Minnesota In an analysis of 193 strains of Brucella recovered in Minnesota from patients in 1945 and 1946, 13 were of the melitensis variety (41). The human cases from which Br melitensis had been isolated occurred in 4 counties adjacent to Iowa, and involved not only packing-house workers who handled fresh pork, but also 2 farmers who had had direct exposure to aborted material from hogs

Brucellosis in the hog due to Br suis itself is difficult enough to control without the added burden of melitensis infections Suis infections in hogs may produce no discernable symptoms to direct attention to the presence of the disease in a herd Abortion may never occur (37) Several studies of experimental and natural infections have clearly demonstrated a remarkable resistance on the part of hogs to Brucella (6, 21) In one experiment just completed at the

The natural and relative resistance of man to Br. abortus is indicated by the frequency with which invasion of the tissues occurs in the absence of any symptoms of disease. In a significant sample of the adult population of Minnesota studied in the Outpatient Clinics of the University Hospitals (66) considerable evidence of subclinical infections was found (Table V) Of 533 consecutive and unselected patients, 104 (19 5%) gave positive reactions upon the intradermal injection of a purified protein derivative of Brucella (48) Only 6 of the positive reactors had a serum agglutinin titer greater than 1:80 and only 1 had an unequivocal diagnosis of brucellosis established by isolation of the organism (Br. abortus) It is highly probable that these data are applicable almost entirely to the abortus problem, in view of the preponderance of bacteriologically identified infections due to this strain in Minnesota

TABLE V

Results of Skin Test Survey with Brucella Antigen (Purified Protein) and Correlation with Agglutinin Titers on 533 Unselected Patients in Adult Outpatient Clinics at University of Minnesota, 1945

Total number consecutive intradermal tests	533
Total number with positive tests	104
Per cent with positive tests	19 5
Results of agglutination tests on 97 of 104 patients having positive intradermal tests	
Total number having positive agglutination test with titers up to 1.80	6
Total number having positive agglutination test with titers higher than 1·80	17

The resistance of most members of a family to outspoken exposure also illustrates this principle Usually active disease develops only in a single member of such a family, although multiple infections are not rare. The family of F H, a woman with severe clinical symptoms and bacteremia, is a typical example (Table VI) Abortions occurred in her father's herd of cattle both before and after her entry to the University of Minnesota Hospitals The animals gave positive reactions to tests for Bang's disease. Accordingly, 8 other members of her family were studied, and 6 showed evidence of having had a subclinical infection with no symptoms at any time

TABLE III

Species of Brucella Isolated from 54 Cases at the University of Minnesota Hospitals

Brucella species	No of cases
Br abortus	50
Br suis	2
Br. melitensis	2

Morales-Otero (47), for example, fed live cultures of Br. abortus to numerous human volunteers and failed to produce a demonstrable infection in any of the subjects He finally succeeded only by the oral administration of 7 consecutive 24-hour broth cultures of a virulent bovine strain The attack rate of naturally occurring clinical infections through ingestion is fortunately not considerable. This is strongly implied by the studies of Fitch and Bishop (23) who demonstrated Br. abortus in the raw milk of 17 of 67 dairies (25 4%) selling milk to a single municipality in Minnesota Such widespread dissemination in raw marketed milk would ravage the populaton if man were not highly resistant to infection by this organism An epidemic occurring in 1946 at Federalsburg, Maryland is one of the few recorded outbreaks of major proportions in humans due to Br. abortus This was described by Steele and Hastings (70); they suggested that Br. abortus can be the cause of an epidemic when the organisms are not diluted by clean milk

TABLE IV

Analysis of Sources of Infection in 54 Cases of Human Brucellosis

Source of infection	No of cases
Oropharynx	
Definitely following ingestion of raw milk (Br abortus)	13
Probably following ingestion of raw milk (Br. abortus)	8
Skin	
Farmers with Bang's disease in herds of cattle (Br abortus)	12
Packing-plant employees (Br suis)	2
Packing plant employees (Br abortus)	12
Respiratory (?)	
Laboratory workers (Br melitensis)	2
Not Known	
(Br abortus)	5

Pathology

General Pathology. Whereas the histopathology in animals has been adequately described in the past (61, 19, 46), this aspect of human brucellosis has been virtually unexplored, due to the low death rate and absence of postmortem material. The often quoted mortality rate of 3 per cent is not founded on accurate data, calculations having been upon highly selected groups of cases Deaths due directly to brucellosis are so rare that only a few careful post-

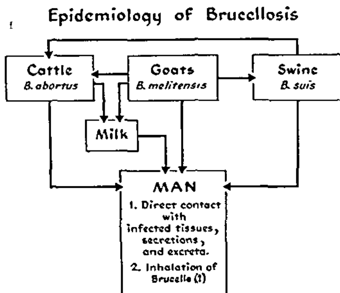


Fig 1 The role of animals in the transmission of brucellosis to man

mortem studies have been performed Up to 1943, for example, the autopsy findings of only 44 cases had been reported, of which only 7 represented fatalities immediately resulting from Brucella infection (45). Perhaps the outstanding finding in any of these fatal cases has been the relative absence of specific lesions comparable to those found regularly and with ease in experimentally infected animals. In the guinea pig, which affords the best example, a granulomatous lesion is seen, resembling that observed in tuberculosis but not identical to it The lesion occurs in various organs, especially in

The skin and oropharynx have been well established as portals of entry by numerous observations in the past It is probable, but less certain, that infections may also occur through the respiratory tract by inhalations of viable Brucella dispersed through such media as dust. Members of the Mediterranean Fever Commission found that the disease could be conveyed to healthy animals by artificially contaminated dust (44). They pointed out, however, that since Brucella cells are easily killed by exposure to sunlight and are diluted rapidly in blowing dust, the transmission by dust under most natural conditions is improbable They suggested that in enclosed areas such as barns, where sunlight is absent and contamination with milk and urine occurs constantly, dust-borne infections could be possible Elberg and Henderson (18) have demonstrated that guinea pigs are nearly as susceptible to Br melitensis and Br. suis infection by the air-borne route as by the subcutaneous route. Human cases have been seen at the University Hospitals in whom the possibility of infection by this route has been seriously considered A summary of the factors involved in the epidemiology of human brucellosis is given in Figure 1.

TABLE VI

Results of Investigations in Family of F. H, Who Was Critically Ill with Active Brucellosis

Relation to Patient	Age	Intradermal test (Brucella *protein*)	Titer of agglutinins	Blood culture	Comment
1. Brother	30	0	1 80	0	—
2 Brother	18	+	1 160	0	—
3. Sister	13	+	1 80	0	—
4 Sister	20	+	1 160	—	—
5. Father	55	+	1:160	0	—
6. Mother	54	+	1.160	0	—
7 Brother	22	0	0	0	Recently released from Army, no exposure
8. Sister	26	0	0	0	Home only weekends, slight exposure

Pathology

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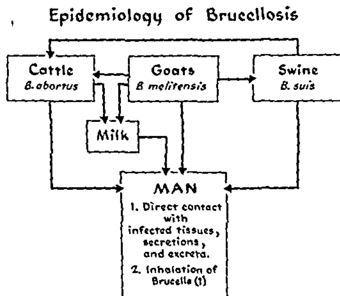


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those with prominent reticulo-endothelial elements; necrosis is infrequently observed in the center of the nodules following inoculation with Br. abortus, but widespread necrosis with suppuration is characteristic of infections with Br suis and Br. melitensis Using these experimental findings as criteria, many investigators have examined the occasional postmortem human tissues for corresponding lesions and have sometimes noted a granuloma with similar characteristics Wohlwill (74) found these in the bone marrow, lymph nodes, and spleen, but not in the liver. In view of present-day knowledge of the consistent presence of granulomas in the liver, their absence in earlier reports is very striking. In an extensive review in 1932, Sharp (59) emphasized that the liver failed to show these cellular aggregations Rabson (50) described a granuloma in the liver of an unproved case of probable bovine origin in 1938, and Forbus (24) mentions having seen them in routine autopsy material which he had not reported. Reviews dealing with similar scattered descriptions only serve to emphasize the paucity of available material in the past.

Recently, at the University of Minnesota, the use of a simple biopsy technic in patients with brucellosis has proved to be a highly successful approach to this problem By examining sections of bone marrow and liver of patients with clinical and laboratory evidence of active infection, considerable insight into the nature of the pathologic processes has resulted Definite histologic changes have been demonstrated in the vast majority of bacteriologically proved cases in which such sections have been examined

In the bone marrow, the fundamental lesions have consisted of granulomas composed primarily of epithelioid cells (71) In some cases these cells have been observed to form almost sheetlike aggregates, with a variable number of associated lymphocytes and only occasional giant cells Variations from this have been seen, differing in the number and character of giant cells as well as the distribution and type of nonepithelioid cell Thus, in one lesion the epithelioid and giant cells were abundantly surrounded by lymphocytes and other mononuclear cells including reticulo-endothelial cells with irregular cell outlines In another, neutrophils were numerous but diffusely arranged, in contrast to those seen in miliary abscesses Eosinophils have also been a prominent constituent of some granulomas, and other features such as great vascularity with numerous

capillaries have been present. A network of reticular fibers has been shown in nodules stained by silver impregnation, but fibrosis has been absent.

In the sections obtained by liver biopsy, essentially the same granulomas as in the bone marrow have been found repeatedly (67). These have been located anywhere in the lobule, and in the portal spaces. In addition, a nonspecific hepatitis has been frequently present with lymphocytic infiltration of the portal spaces as well as diffusely throughout the lobule. Caseation has not been observed in the liver granulomas nor in those in the bone marrow It is to be emphasized that the cases studied were infections due to Br. abortus. Special attempts to demonstrate the organism in the lesions have failed despite successful isolation of Brucella from cultures of venous blood and aspirated sternal marrow.

One of the most striking features of these lesions is the ease with which they have been found in essentially every active infection. A single examination of a tiny portion of bone marrow or liver has proved sufficient in several instances for their demonstration From this fact, it can now be concluded with assurance that brucellosis is a disease which produces widespread granulomatous lesions in various human tissues.

Intracellular Parasitism Additional understanding of the pathogenesis of brucellosis has been provided by Meyer (45), who reported the first recorded example of intracellular parasitism by this organism in an infected person This phenomenon had been previously described in animals by Fabyan (19) who observed it in guinea pigs, and by Smith (63) The latter worker demonstrated large numbers of Br abortus in the cytoplasm of chorionic cells in the placenta of aborting cows In 1937, Goodpasture and Anderson (27) found that Br abortus entered and proliferated within both ectodermal epithelial cells and mesodermal connective tissue cells following inoculation of the chorioallantoic membrane of chick embryos. He also witnessed the formation of nodules by proliferated fibroblasts which contained the bacilli in their cytoplasm Buddingh and Womack (7) made similar studies with all three strains, and concluded that Br abortus and Br suis showed a preference for mesodermal cells, in contrast to Br melitensis which could only be found in ectodermal cells

Meyer's patient was a laboratory attendant who succumbed after

three weeks to an infection resulting from the ingestion of a broth culture of *Br. suis* Intracellular parasitism was noted in the epithelial cells of both Bowman's capsule and the proximal convoluted tubules Attempts to demonstrate the intracellular presence of Brucella organisms in the tissues of three patients at the University of Minnesota were unsuccessful, although extracellularly the organisms could be easily detected in sections of a mitral valve leaflet from which they had been previously cultured (Figs 2, 3) Ruiz-



Fig 2. Brucella endocarditis Subendocardial location of Br abortus in leaflet of mitral valve (× 100) Nyka stain



Fig 3 Brucella endocarditis High power magnification (× 600) of section shown in Figure 2 Nyka stain.

Castaneda (54), encountering similar difficulties with human tissues, investigated this problem in guinea pigs and rabbits; he succeeded in locating parasitized parenchymal cells in the liver, spleen, and testicle He was also able to show phagocytosis by many polymorphonuclears and macrophages He advanced the hypothesis that the cytoplasm is used as a source of material for bacterial growth and that the intracellular location provides a means for preservation of the organisms The suggestion was also made that nodule formation resulted from a cellular reaction of monocytes, polymor-

phonuclears, and lymphocytes around wandering macrophages carrying Brucella

Special Pathology. From time to time, various localized disorders involving a single organ or structure have been noticed. As these have been fully described and discussed elsewhere, a detailed review will not be given here except for those examples which contribute directly to the elucidation of the general pathologic process From this point of view, the most remarkable of the focal lesions are those involving endocardium At least 6 cases of endocarditis have been reported which have been substantiated by bacteriologic and anatomic evidence at autopsy. The first was that of Casanova

TABLE VII

Summary of Reported Cases of Brucella Bacterial Endocarditis Substantiated by Bacteriologic and Anatomic Evidence at Necropsy

Author	Brucella	Site of vegetation	Previous pathology in affected valve
Casanova and d'Ignazio (9)	melitensis	Aortic valve	None
Rothman (51)	melitensis	Aortic valve	None
Spink and Nelson (64)	abortus	Aortic valve	None
Smith and Curtis (62)	abortus	Aortic valve	Probably rheumatic
Spink *et al* (65)	abortus	Mitral valve	Rheumatic
DeGowin *et al* (12)	suis	Mitral valve, aneurysm of femoral artery	None

and d'Ignazio (9) in 1933; it was due to Br melitensis, and there was involvement of the aortic valve. This case and those reported since then are summarized in Table VII It can be seen that a previously damaged valve is not a prerequisite for the development of a vegetative endocarditis by any of the strains, and that both mitral and aortic valve leaflets may become involved (Figs 2–3) The generalized anatomic findings are similar to those seen in bacterial endocarditis due to Streptococcus viridans (2) A myocarditis has occurred which is characterized by diffuse and focal infiltrations of monocular cells, some of which are said to have the appearance of Aschoff lesions (65) In the kidney, a proliferative type of

glomerulonephritis may be prominent. Infarcts appear in the spleen and other organs and evidence of passive congestion of the lungs and liver are also present A very significant feature of the cases of fatal endocarditis is the almost universal absence of granulomatous lesions in the various organs Only a few were present in the kidneys of the case reported by DeGowin *et al.* (12) but were entirely lacking in the 2 cases reported by Spink *et al.* (64, 65). Skin tests with the usual Brucella antigens in 4 cases observed at the University of Minnesota and in the 1 reported by DeGowin *et al.* were all negative. It has been suggested that the granulomas represent a desirable defense reaction by the tissues and that they fail to develop in fatal infections in which the resistance of the host is overwhelmed. The matter of hypersensitivity as well as treatment in this condition are discussed at greater length in other sections

Brucella infections have been reported to be associated with another fatal condition, that of Hodgkin's disease In a series of cases studied at Duke University by Wise and Poston (73), Br. melitensis or Br. suis (but not Br abortus) were isolated from the blood or lymph nodes of 14 patients with Hodgkin's disease. Because these individuals were residents of an endemic area, a corresponding survey was made of a group of 67 controls with other diseases of the lymph nodes, only a single culture yielded Brucella Although no claim was made by the authors for an etiologic relationship between Hodgkin's disease and brucellosis, they felt that this possibility deserved some consideration Along this line, Forbus (24) has attempted to show parallel features in the histologic pattern of brucellosis and Hodgkin's disease

Certain common properties appeared to be present in all the strains isolated in the Hodgkin's cases For one thing, they were found to possess little pathogenicity for guinea pigs. Thus, it was not only impossible to reproduce Hodgkin's disease experimentally, but there was also considerable difficulty encountered in attempts to infect the animals and produce any anatomic changes (25). Another interesting feature was the lack of immune response in the Hodgkin's group of infected persons None of those inoculated intradermally with Brucella antigens demonstrated positive skin tests and many showed an absence of agglutinins in their serum

Brucella organisms have also been shown to cause localized disease of bones, the central nervous system, the gallbladder, and the genitalia. In addition, there has been some evidence that diseases of the eye and lungs may result from Brucella infection

Infection of bones may involve the spine, long bones, and pelvis. The existence of Brucella spondylitis associated with pain in the back has been demonstrated roentgenographically in humans A few cases, such as that of Hardy's (34), have been described in which Brucella have been actually isolated at surgery. In Hardy's patient, Br. abortus was cultured from a cavity located anterior to lumbar vertebral bodies This patient, incidentally, developed a psoas abscess about 9 months after spinal fusion. Because there has been little opportunity to observe directly the pathologic process in patients, much of our knowledge of this disorder must be based on the excellent descriptions presented by Feldman and Olson (22) on spondylitis in hogs. They found lesions consisting of small irregular cavities filled with thick, creamy, odorless material in the intervertebral disks of the lower spine These were observed to extend into the vertebral bodies as well as into the spinal dura On the basis of experience with a number of patients at the University of Minnesota Hospitals, it appears that the prognosis is favorable and that uneventful recovery may be expected without surgical intervention. In the long bones (43) and pelvis (42), a chronic low grade osteomyelitis has been found

In contrast to chronic disease of bone, chronic arthritis is rarely, if ever, due to Brucella infection Not only has it been impossible to establish an etiologic relationship by isolation of the organism, but it has been found in a careful study by Darley and Gordon (11) that the incidence of Brucella sensitization is no greater in a large group of arthritics than in a control group with no symptoms Acute or intermittent joint effusions do occur, but chronic and deforming disease of an arthritic nature is probably a very rare manifestation of brucellosis.

In 1934, DeJong (13) reviewed the findings and outcome of 11 verified cases of brucellosis with involvement of the central nervous system; 3 proved fatal, and the others, of varying severity, included a few which were quite mild clinically. Physicians have always been

impressed by the prominence of nervous symptoms in brucellosis Complaints such as headaches, mental fatigue, depression, and fleeting, ill-defined aches have suggested the possibility that nervous system involvement is much more common and more widespread than it has been possible to demonstrate With this in mind, attempts were made at the University of Minnesota Hospitals to isolate Brucella from the cerebrospinal fluid of patients with brucellosis having minimal neurologic symptoms. In one such patient Br abortus was obtained on two occasions by Dr. W. H Hall following injection of the fluid into the allantoic sac of chick embryos after routine cultural methods failed to yield any organisms. Other tests disclosed no abnormalities of the spinal fluid Continued studies of this type, as well as careful postmortem examinations of the brain, may disclose a higher incidence of nervous system involvement than previous work has shown In this country, the case described by Hansmann and Schenken (31) and also by Sanders (57) is the sole report of meningoecenphalitis with autopsy findings due to Brucella This patient died 13 months after onset of symptoms from rupture of a mycotic aneurysm of the basilar artery. There was thickening of the leptomeninges due to a chronic inflammatory process, and a heavy infiltration of the adventitia of the meningeal vessels In the brain, collars of lymphocytes surrounded many blood vessels, and inflammatory cells were present in the perineurium of the nerve roots.

None of the numerous papers dealing with pulmonary changes in brucellosis has conclusively established that the lungs may be involved in this disease In rare instances, Brucella organisms are said to have been isolated from the pleural fluid or the sputum, but for the most part the main evidence has consisted of low agglutinin titers or positive intradermal tests Pulmonary disease has not been noted in over 90 proved cases of brucellosis at the University of Minnesota

There is little conclusive evidence that diseases of the human eye may be due to brucellosis Woods (73) has encountered cases of iritis, choroiditis, and keratitis in patients whose histories, skin tests, or agglutinin titers have suggested the possibility of previous Brucella infection He has also described favorable therapeutic

results with Brucella antigens in some of these patients. However, the diagnosis was not verified by culture in his reported cases

Diagnosis

Clinical Findings Various authors who have analyzed the clinical picture of brucellosis have added little to the classic description of Hughes (35), published in 1897. Now, as then, the clinical picture is that of a febrile disease of short or long duration marked by weakness, a variety of aches, nervousness, few physical findings, and a tendency to relapse During the acute phase and during relapses

TABLE VIII

Analysis Localizing Physical Abnormalities in 54 Cases of Human Brucellosis

Manifestations	No of cases
Acute cases	
Hepatomegaly	2
Splenomegaly	4
Skin eruption	1
Lymphadenopathy	7
No abnormality	11
Total	*23*
Chronic cases	
Lymphadenopathy	15
Hepatomegaly	4
Splenomegaly	15
Subacute bacterial endocarditis	4
Spondylitis	4
Encephalitis	1
Pericholecystic abscess with liver abscess	1
Destructive sacroiliac arthritis	1
No abnormality	8
Total	*32*

these manifestations may be sharply defined with high fever, rigors, and profuse sweats Yet the degree of prostration during such bouts may be remarkably slight, and it is not unusual for persons so afflicted to be ambulatory Qualitatively, the subjective disturbances during intervals between or following these attacks may be identical, though far less pronounced Weakness is an invariable complaint during all stages, and its persistence is largely responsible for the discouragement that occurs in chronically ill patients whose

strength is inadequate for the pursuit of necessary and customary activities Such discouragement may lead to severe emotional depressions, with suicide a serious possibility.

The relative scarcity of physical findings in both the acute and chronic stages is illustrated in Table VIII, which was compiled from cases at the University of Minnesota. The etiology in each of these was established by isolation of the organism. In this analysis, the acute period was arbitrarily designated as that terminating after 3 months of symptoms. An illness continuing beyond 3 months was regarded as chronic. It can be seen that the two most frequently encountered findings were lymphadenopathy, occurring in slightly less than 50 per cent of patients, and splenomegaly in about one-third of them Not included in the table are two findings which have also been observed rather often. They appear to represent a vasomotor disturbance of the extremities and consist of sweaty palms and a bluish mottling of the skin of the arms and legs.

An unusual opportunity to study the clinical findings in acute cases among laboratory workers developed during the war at Camp Detrick, Maryland, where 17 persons contracted infections due to either Br suis or Br melitensis (32). The manifestations here were even less numerous than those present in the Minnesota group of cases. In only a single patient was the spleen palpable Otherwise no abnormalities were noted except for herpes febrilis in 2 cases and abdominal tenderness in 2. The duration of fever averaged 95 days, and the average length of illness in 10 patients was slightly less than 6 months

Subclinical Infections Not only may abnormal physical findings be lacking, but symptoms may be entirely absent, even in persons with bacteremia Such active subclinical infections were detected over 40 years ago by E A Shaw (60), a British naval surgeon working with the Mediterranean Fever Commission. He isolated Br. melitensis from the blood or urine of 10 dockyard workers engaged in hard physical labor in Malta Similar instances of subclinical infections in epidemics have been described by Huddleson (33) and Jordan and Borts (39), and by Ruiz-Castaneda (55) in asymptomatic patients after clinical recovery from active infection Brucellosis is not the only disease to show subclinical infection, as may be

seen by comparison with other bacterial infections, such as typhoid fever

"Chronic" or "Indolent" Brucellosis In addition to the undoubted infections and the subclinical infections, a third clinical entity of questionable validity has been proposed and perpetuated This debatable condition is often referred to as "chronic brucellosis," but is perhaps more aptly entitled "indolent brucellosis" (11). Persons so afflicted are said to suffer from symptoms of malaise, headache, sweating, muscular aches, less than a degree of fever, insomnia, sexual impotence, marital strife, joblessness, and idleness often resulting in weight gain

Such patients are the despair of some physicians Their gloomy countenances repeatedly confront the conscientious physician who cannot demonstrate an objective sign of disease The patient appears well nourished and even robust. He gives no distinct history of clinical infection. Laboratory tests, including the agglutination test and blood culture, are negative; or, to make matters worse, he may have only a barely detectable titer of ±1 40. Such is the plight of the practitioner whose diagnostic acumen and professional conscience are strained to the utmost.

Unfortunately, because of its obscure nature, this problem lends itself with the utmost difficulty to careful investigation. One impressive study has been performed to determine whether "chronic," "indolent," or "allergic" brucellosis exists as a definite disease Using a careful statistical approach, Darley and Gordon (11) found a higher incidence of positive skin tests with brucellergen in chronically ill patients than in asymptomatic controls. Complaints similar to those mentioned above were present in the sensitized patients as a group, but not necessarily in the individual patient. They concluded, therefore, that "indolent brucellosis" was a distinct clinical condition, but that its characteristics were too indefinite to be of practical value in the consideration of the individual patient Until this syndrome is fully verified, and until the individual case can be identified with certainty, the dispensing of empiric and doubtful remedies must be curtailed

Laboratory Diagnosis Although the clinical picture and several laboratory methods are helpful, the diagnosis of brucellosis can only

be made with assurance by isolation and identification of the organism. As Brucella are a fastidious group in their cultural requirements, certain well-known environmental and nutritional conditions must be supplied for optimum results. Of the three strains, Br. melitensis and Br. suis grow in ordinary atmosphere but Br. abortus requires a carbon dioxide tension approximately 10 per cent above that of air Several mediums provide the factors permitting good growth. Those enriched with liver or potato are satisfactory. Convenient mediums to prepare are Bacto-tryptose broth and Bacto-tryptose agar, manufactured by the Difco Laboratories

In routine practice, only the blood is cultured. A practical method is to transfer 5 cc directly from the patient to a 3 ounce rubber-stoppered bottle containing the tryptose broth with 1 per cent citrate. Carbon dioxide is added, and after incubation for 5 days subcultures are made to tryptose agar slants, where colonies appear within 48 to 72 hours. With this method, positive cultures have been obtained at the University of Minnesota in approximately a third of the patients seriously suspected of having active brucellosis on the basis of clinical and laboratory evidence

An ingenious and very convenient method for blood culture has been recently introduced by Ruiz-Castaneda (52). By merely introducing a layer of agar against one wall of the bottle containing the broth, he has devised a scheme which permits subcultures to be made simply by spreading the mixture of blood and broth over the agar for an instant (Fig 4) Contaminants grow out on the agar in less than 48 hours and Brucella colonies appear after that time. They are readily recognized through the thin transparent layer Such means of subculturing without exposing the culture to air have the obvious advantages of eliminating a source of contamination and also of protecting the technician from possible infection. The appearance of typical Brucella colonies on the agar alerts the person handling the culture to the need for proper care in subsequent work with it At the University of Minnesota, this technic was adopted shortly after its introduction and has been in use for over a year After considerable experience with the double medium, it has been found that although it is generally very satisfactory, some shortcomings must be expected At first, it was noticed that

the agar sometimes softened and became separated from the glass wall. This was overcome by increasing the amount of agar in the solid medium from 3 to 3 6 per cent. It was also noticed that the colonies of Brucella on the agar were easily washed off the surface into the broth, after which growth on the agar could not be reestablished. Hence, one must handle the bottle with extreme caution during the process of transferring colonies for further identification.



Fig 4 Double media Note vertical layer of agar on side

There appears to be little gain in the routine culture of other fluids than blood In three patients, Brucella were isolated from aspirated bone marrow when cultures of blood obtained at the same time remained negative These are exceptional circumstances, however, and do not warrant bone marrow culture in every case. Attempts to culture urine and bile have proved disappointing. In cases with symptoms referable to the central nervous system, it is essential that cultures of the spinal fluid be performed. A technic

which is well adapted to this purpose is the inoculation of chick embryos. By injecting the spinal fluid directly into the chorioallantoic sac, Brucella were isolated from 2 of our patients, one of whom had clinical symptoms of encephalitis. In both instances, the organism could not be isolated in tryptose broth.

In the absence of positive cultures, the presence of agglutinins in significant titer is the best laboratory evidence of active infection In spite of reports to the contrary, we are firmly convinced that brucellosis can be eliminated as a diagnostic possibility on the basis of a negative agglutination test except during the initial days of the acute infection and in a rare case of chronic disease. In over 90 cases of culturally identified Brucella infections at the University of Minnesota, there has never been an instance in which agglutinins failed to appear in the serum. Reports by Ruiz-Castaneda (55), as well as Boak and Carpenter (26) described the absence of agglutinins in 6 to 12 per cent of cases verified by blood culture. It is felt that several factors may be responsible for such results One possibility, already referred to, is that these instances represent exceedingly early infections in which agglutinins eventually occurred within the expected period of time. Another factor is the use of an unreliable antigen in the performance of the test. Judging from a study made by Eisele and co-workers (16), a wide discrepancy exists in results obtained with various antigens in different laboratories. They found, for example, that of 18 serums reported negative by one or more laboratories, 16 were called positive in titers of 1 200 or higher by other laboratories. The need for the general employment of a well-standardized antigen can be clearly appreciated from this information.

Another source of false negative agglutination tests is the failure to note the occurrence of a prozone phenomenon This has been emphasized by Griffitts (28) in a careful investigation of the immune properties of serums from persons with positive histories of brucellosis and an absence of serum agglutinins by ordinary methods In several of these serums he found a zone of agglutination occurring in only one or a few consecutive dilutions of intermediate titer with no agglutination present above or below those levels For example, in serum from a patient designated RC, ag-

glutination was present only in dilutions of 1:64 and 1:128 Of perhaps greater interest was his discovery that all serums tested from such persons possessed agglutinin blocking properties. This corresponds to an effect observed by Weiner (72) in the serums of Rh-negative mothers bearing infants with erythroblastosis foetalis. The serums of such individuals, with obvious clinical evidence of Rh sensitization, possessed no anti-Rh antibodies by the usual tests, but these same serums prevented the agglutination of Rh-positive red cells by known agglutinating serums In the case of agglutinin-free serums from persons known to have been infected with Brucella, Griffitts found a similar blocking effect when such serums were incubated with antigen an hour before agglutinin was added. He further noted that this property depended on a labile factor which could be reduced by heating at 56 C. for 15 minutes.

Finally, he demonstrated that the blocking serums could be shown to agglutinate antigen when rabbit serum was substituted for saline as the diluent. Titers as high as 1:256 resulted from this technic after failure of the saline dilution method to produce any essential agglutination

In view of the findings of Griffitts and of Eisele *et al.*, as well as our own experience with verified cases, it can be stated with fair assurance that the failure to demonstrate agglutinins in a patient with brucellosis reflects an incomplete or improper search for such antibodies rather than an absence of immune response by the infected individual This failure can be avoided in the vast majority of instances by the use of proper materials, especially a well-standardized antigen, and by careful technics which provide for the detection of a prozone. In a small number of cases yielding negative tests under the best conditions, it may be necessary to resort to the special methods described by Griffitts

Although experience has shown that a positive agglutination test in significant titer ranks second to positive cultures as the most reliable laboratory evidence of active brucellosis, it is not meant to imply that its shortcomings are not numerous Emphasis is given to the use of the term "significant" in place of "diagnostic" At its best, the former expression has only loose application; the higher the titer, the greater is its significance No precise level can be stipulated, even though it may be stated that the lowest value observed

in the bacteriologically verified cases at the University of Minnesota has been 1:80 It will be shown below that in several conditions without active Brucella infection, higher agglutinin titers than this may be present (Table IX). The importance of the agglutination test depends entirely on the confirmatory information it offers when viewed as one link in the entire chain of evidence.

The pitfalls in the interpretation of the test are as numerous as the conditions which may be accompanied by positive titers in the absence of active Brucella infection (Table IX) Perhaps the largest of such categories consists of the numerous individuals who have

TABLE IX

Conditions Other Than Active Brucellosis in Which Serum Agglutinins for Brucella May Be Present

Condition	Highest agglutinin titer observed
[illegible]	1.2,560
[illegible]	1:1,280
[illegible]	1·2,560
[illegible]	Low
Typhoid fever (3)	1·160
Anamnestic reactions (3)	1:160

recovered from clinical or subclinical infections. In studying the natural course of the disease in animals, it has been found that 12 months after the initial inoculation with Br abortus no evidence of active disease can be found by careful examination of all tissues Yet agglutinins in high titer are still demonstrable in the serums of guinea pigs (Table X) Similarly, in patients treated with combined streptomycin and sulfadiazine therapy, there is a persistent elevation of titer as long as a year following apparent cure. Such treated individuals have been carefully checked for evidence of continued bacteremia as well as clinical signs of infection. It can certainly be concluded that agglutinin titers remain elevated long after the clinical activity of the disease has ceased

It is well known that the introduction of Brucella antigens into the tissue, either for skin testing or therapeutic purposes, stimulate the production of agglutinins (5, 26) It is now known that inocula-

tion with cholera antigens has the same effect Eisele and associates (17) found, in a survey of 100 ex-servicemen who had received cholera vaccination, that 56 per cent had positive Brucella agglutination tests in titers of 1:20 or higher. These were found to persist in 27 per cent for periods longer than 18 months In a subsequent study in which 20 persons were given the standard cholera immunization, Brucella agglutinins developed in every subject, rising to 1:320 or higher in 45 per cent (15) Other instances in which the cross agglutination phenomenon for Brucella occurs are typhoid fever (3) and tularemia (34) In addition, Brucella agglutinins may rise in response to an anamnestic reaction stimulated by another infection Borts (3) mentions a rise to 1:160 occurring in pneumococcic pneumonia

TABLE X

Agglutinins in Serums of Untreated Guinea Pigs After Apparent Complete Recovery from Experimental Infections with Br abortus, one year after inoculation

Animal No	Skin test	Blood and tissue cultures	Lesions	Agglutinins
3755	0	0	0	1·1,280
3744	0	0	0	1:640
4129	0	0	0	0
3742	0	0	0	1 640
4982	0	0	0	1 160
3730	+++	0	0	1 1,280
3750	+++	0	0	1 1,280

Of all the special tests applicable to the diagnosis of brucellosis, the skin test is undoubtedly used most widely because of its simple technic Furthermore, it suffers the most widespread abuse The problem of brucellosis has been greatly confused as a result of the unjustified inferences drawn by many individuals from dermal reactions to Brucella antigens.

In a study of this subject at the University of Minnesota (5), a critical survey of the properties of representative antigens was made and their practical significance as a diagnostic device was evaluated. For purposes of comparison, three Brucella fractions were applied to the skin in dosages of 0 01 mg each. Included in this group were a polysaccharide fraction, a purified protein derivative, and the nucleoprotein, brucellergen In addition, a suspension of heat-killed

Brucella cells was used. As a result of testing more than 250 subjects by the simultaneous injection of these agents, it was possible to compare their merits and shortcomings under a variety of circumstances

None of the information afforded by the study justified the designation of a satisfactory skin-testing agent from this group of substances Almost all possessed disqualifying deficiencies. Brucellergen elicited too many violent reactions (10%). The polysaccharide and vaccine produced agglutinins which would confuse the diagnostic picture. The purified protein of Morales-Otero (48) gave most promise of being the antigen of choice, since it appeared to be highly specific and provoked only infrequent severe local reactions Some question still remains, however, as to its property of stimulating antibodies

Far more important than the study of the specifications of a satisfactory antigen, however, is the evaluation of its practical significance as a diagnostic device (Table XI). It is immediately apparent

TABLE XI

Per cent Incidence of Brucella Hypersensitivity Elicited by the Simultaneous Application of Four Representative Skin Test Antigens

Individuals with positive cultures	100
Individuals with positive agglutination tests	94
Individuals with negative agglutination tests	42
Individuals with negative agglutination tests, negative cultures and no history of exposure to Brucella infection	17

from the high incidence (17%) of dermal sensitivity found in persons with no signs of brucellosis that a positive test is not peculiar to active infection Nor can the intensity of reaction be used as a guide, for it was learned that in active infections weak reactions are as frequent as strong In severe infections, dermal sensitivity may be absent A positive test implies only the acquisition of hypersensitivity through previous exposure to the organism and nothing can be inferred relative to the status of infection on the basis of the test alone. In endemic areas, where the problem is of most importance, and where it is difficult to escape exposure, a positive test has no special diagnostic significance

One of the uses advocated for the skin test has been to detect those

active cases alleged to have absent agglutinins. This subject has been discussed at length earlier and it has been pointed out that such cases rarely occur. Also, the use of the skin test in detecting "Brucella allergy" has been dealt with in considerable detail and our views in this matter have been given. Probably the only valid use for this test is in those instances where the possibility exists of cross agglutinins from another infection or from cholera vaccination Eisele and associates (15) have shown that the brucellergen test remained negative in the 20 persons who received the full course of cholera vaccine Hence, a negative skin test might serve as significant evidence in identifying cross agglutinins. At the University of Minnesota, the Brucella skin test has been abandoned as a routine diagnostic aid.

From the theoretic point of view, a number of interesting observations have been made with the carbohydrate fraction* of Brucella The properties of the polysaccharide antigen are: (*1*) immediate formation of wheal and erythema, (*2*) reaction suppressed by benadryl; (*3*) agglutinin production induced in 50 per cent of individuals, (*4*) passive transfer of sensitivity; (*5*) positive reaction in subacute bacterial endocarditis due to Br abortus; (*6*) no violent reactions with 0 01 mg skin test doses. Special consideration is directed to the fact that this agent was found to produce a positive test in two patients with bacterial endocarditis due to Br abortus This is in contrast to negative results obtained in these cases with the conventional Brucella antigens which produce the delayed type of reaction It is also noteworthy that upon retesting these patients after successful specific therapy, all antigens gave positive reactions.

Two additional immune reactions have been employed to assist in diagnosis One of these, the complement-fixation test, has been found to possess no advantage over the agglutination test which is more easily performed The test for opsonins, however, still has many adherents According to Huddleson (34) and Harris (29), it is often possible to ascertain the status of infection or noninfection by the opsonocytophagic power of blood and its relationship to the skin test and agglutination test For example, low phagocytic activity in the presence of positive agglutination and skin tests is said

* This material was obtained from the Lederle Laboratories in Pearl River, New York.

to be diagnostic of active infection Our results with this test have been so inconsistent that it is no longer employed at the University of Minnesota However, the prognostic significance of the opsonic index needs further investigation.

The fact that the leukocytes rarely number over 10,000 per cu mm. in active brucellosis, is of some auxiliary value in diagnosis Associated with this is a relative or absolute increase in the number of lymphocytes. The sedimentation rate may be normal or elevated If elevated, the activity of the disease can be gaged sometimes by the rapidity with which the sedimentation rate returns to normal

Treatment

The most important advance in the study of human brucellosis has been the successful use of specific antibrucella agents Until these agents could be used for human infection, a number of therapeutic procedures were developed whose effects depended on a change of the immune state by the administration of preformed antibodies in immune serums, or by influencing the degree of hypersensitivity with appropriate antigens This review is concerned only with specific antibrucella agents, such as the sulfonamides and antibiotics

At the present time, the specific treatment of human brucellosis can be accomplished by the simultaneous administration of streptomycin and sulfadiazine The events culminating in the choice of this successful combination form an instructive chapter in the history of antibiotics and their application to human infection. Aureomycin, one of the newer antibiotics has also produced excellent results Its effectiveness cannot be fully evaluated, however, until the treated cases have been observed for a longer period of time.

Experimental Studies At the University of Minnesota, a method was devised for the rapid screening of various agents which showed promise of effective antibrucella activity. This was based on the demonstration by Goodpasture and Anderson (27) that chick embryos could be readily infected with Brucella Accordingly, the drug to be studied was evaluated on the basis of its ability to protect the chick embryo from lethal infection and to eliminate viable Brucella from its tissues This method, described in detail elsewhere (30, 58a), has the advantages of speed and numbers A great many

infected eggs may be conveniently tested within a period of 2 weeks under the conditions employed.

The first group of agents to be evaluated comprised the sulfonamides, including sulfanilimide, sulfapyridine, sulfathiazole, sulfadiazine, and sulfamerazine. Each of these drugs prolonged the survival rate of infected chick embryos as compared with nontreated controls, but the eradication of viable Brucella organisms from the tissues was incomplete Penicillin was also tested and found to provide little, if any, protection

Following the report by Jones *et al.* (37) that chick embryos infected with Brucella could be protected with streptomycin, this antibiotic was also evaluated, and the results obtained were similar to those found with sulfadiazine. When these two agents were used in combination, however, they were found to exert a synergistic effect (58b) in protecting the infected embryo The survival rate after the combined therapy was much higher than with either drug alone, but more important, the number of Brucella organisms isolated from the tissues was very greatly reduced A summary of these observations is given in Table XII

TABLE XII

Protective Action of Antibiotics Against Brucella Infection in Chick Embryo

Antibiotic	Survival rate of embryos	Eradication of Brucella organisms from tissues
Penicillin	No increase	None
Sulfadiazine	Increased	Slight
Streptomycin	Increased	Slight
Streptomycin + sulfadiazine	Markedly increased	Marked

Clinical Studies As each new drug became available it received clinical trial in the treatment of brucellosis With the sulfonamides, scattered case reports gave conflicting evidence of their value This was borne out in the variable results obtained in the treatment of 20 cases at the University of Minnesota Sulfanilamide was used in 4, sulfamerazine in 1, and sulfadiazine in 16 of these patients. Of the 20, only 6 recovered completely, 13 showed some improvement, and

to be diagnostic of active infection. Our results with this test have been so inconsistent that it is no longer employed at the University of Minnesota. However, the prognostic significance of the opsonic index needs further investigation.

The fact that the leukocytes rarely number over 10,000 per cu. mm. in active brucellosis, is of some auxiliary value in diagnosis. Associated with this is a relative or absolute increase in the number of lymphocytes. The sedimentation rate may be normal or elevated. If elevated, the activity of the disease can be gaged sometimes by the rapidity with which the sedimentation rate returns to normal.

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The most important advance in the study of human brucellosis has been the successful use of specific antibrucella agents. Until these agents could be used for human infection, a number of therapeutic procedures were developed whose effects depended on a change of the immune state by the administration of preformed antibodies in immune serums, or by influencing the degree of hypersensitivity with appropriate antigens. This review is concerned only with specific antibrucella agents, such as the sulfonamides and antibiotics.

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TABLE XIII

Summary of Results with Sulfadiazine–Streptomycin Therapy of Brucellosis Due to Br. abortus at the University of Minnesota

Period of observation	2–14 months
Total number of cases	16
Number with clinical recovery without clinical relapse	15
Number of clinical relapses	1
Number of bacteriologic relapses	3

The results are summarized in Tables XIII and XIV From the point of view of clinical symptomatology, all but 1 patient at the University of Minnesota receiving this treatment recovered without relapse during periods of observation ranging from 5 months to over a year. However, 3 patients experienced bacteriologic relapses, as demonstrated by isolation of the organism after completion of therapy; of these, only 1 suffered an associated recurrence of symptomatology. The survival of these organisms after therapy does not appear to be on the basis of reduced sensitivity to streptomycin All three strains were tested before and after treatment and in each case the sensitivity had not been found to change The production of clinical cure without bacteriologic cure is a puzzling phenomenon which still requires explanation It undoubtedly represents a favorable alteration in the balance between host resistance and bacterial virulence, and can be compared to untreated instances of subclinical infection already discussed An important approach to this problem will be a comparison of the virulence for experimental animals of these organisms isolated after treatment, with those isolated originally.

Of all the cures produced in this study and others, that effected in

TABLE XIV

Summary of Results Obtained with Sulfadiazine–Streptomycin Therapy of Brucellosis Due to Br melitensis at the Mexico General Hospital, Mexico, D F

Period of observation	2–9 months
Total number of cases	48
Number with clinical recovery without clinical relapse	24
Number of clinical failures or relapses	24
Number of bacteriologic failures or relapses	25

in 1 patient with bacterial endocarditis the course of the disease remained unchanged In 7 of the patients, bacteremia was demonstrated after treatment was discontinued. Hence, as might be expected from the experimental results in the chick embryo, the sulfonamides were only partially effective in the clinical arrest of *brucellosis*

A similar correlation between experimental and clinical results was evident in the case of penicillin. Except in 1 patient, penicillin appeared to possess no therapeutic value in this disease The one exception was a 41 year old farmer who had been totally disabled for nearly a year by the infection Br. abortus was recovered from his blood and found sensitive *in vitro* to 0.1 unit of penicillin per cubic centimeter. He recovered completely after receiving over a million units in 9 days. It is to be emphasized that this patient received amorphous penicillin.

Streptomycin presented a therapeutic enigma. It was found to have disappointing activity clinically against Brucella organisms that manifested marked *in vitro* sensitivity to concentrations which were readily attained in the blood of patients. On the basis of observations in the streptomycin treatment of 45 patients by the Committee on Therapeutics and Other Agents of the National Research Council, and 25 cases studied in the Army, it was felt by Pulaski and Amspacher (49) that the over-all results clearly demonstrated the ineffectiveness of streptomycin in brucellosis This coincided with our results in the streptomycin treatment of 7 patients, of whom none recovered completely.

Combined Therapy Up to the present time, sulfadiazine and streptomycin have been used simultaneously in this clinic to treat 16 cases of brucellosis in whom the diagnosis had been proved by the isolation of Br abortus In addition, we have cooperated with Dr. M Ruiz-Castaneda in administering this treatment to 48 cases due to Br. melitensis at the Hospital General in Mexico City (56) The results in the patients at the University of Minnesota substantiate the observation of earlier reports (14, 49, 68), which demonstrated the therapeutic effectiveness of this combination in patients infected with Br abortus In the treatment of infections due to Br. melitensis, however, *this method has been less successful*

accompanied by less satisfactory results in some cases Vestibular dysfunction has been a serious problem in Mexico City It has been observed in 13 2 per cent of patients receiving streptomycin for infections due to Br. melitensis.

Aureomycin. Twenty-four cases of brucellosis due to Br melitensis have been treated with aureomycin at the Mexico General Hospital (69). This new antibiotic, obtained from the mold Streptomyces aureofaciens by Duggar of the Lederle Laboratories, was substituted for streptomycin in the treatment of these Mexican patients for several reasons. As already mentioned, streptomycin produced less satisfactory clinical results than had been obtained in *Br. abortus* infection in the United States, and the rate of vestibular disturbance was significantly high following its use. Furthermore, the repeated intramuscular injection of streptomycin required hospitalization. Hence, the number of patients who could be given this medication was limited by the shortage of hospital beds in Mexico City. Aureomycin, on the other hand, had produced no serious side effects in patients who had already received it for other illnesses It could be given orally and was therefore appropriate for the treatment of patients in the dispensary or at home

In the first part of the study, 16 patients received both aureomycin and sulfadiazine, in order to determine the effect of substituting aureomycin for streptomycin The immediate results with this combination surpassed those observed with any previous form of treatment used for infections due to Br melitensis. The next 8 patients, therefore, were given aureomycin alone and the results were equally good

Every patient in this series was febrile before treatment, and the diagnosis in each instance had been established by the isolation of Br melitensis from the blood at least once. The illnesses were generally of long duration and in many instances severe. Without exception, prompt clinical improvement occurred after 2 or 3 days of treatment with aureomycin by the oral route. Within this period, the temperature became normal and most subjective complaints disappeared. It was necessary, for the reasons already stated, to treat more than half of the cases on an outpatient basis. These patients appeared to receive as great benefit from aureomycin as those who were hospitalized

a case of Brucella endocarditis is the outstanding testimonial to the specificity of the combined treatment method The diagnosis in this case was based on the isolation from the blood of Br. abortus four times, and on typical clinical signs of subacute bacterial endocarditis with involvement of the aortic valve. A confirmatory sign, already mentioned, was the negative reaction obtained to all intradermal tests except that performed with the polysaccharide antigen Treatment consisted of a total dose of 52 Gm. of streptomycin in 14 days plus 129 Gm of sulfadiazine in 22 days Except for residual vertigo, the patient has remained well for over a year. Blood cultures have remained negative, marked dermal hypersensitivity to all Brucella antigens has developed, and the intensity of the aortic murmur has decreased greatly.

Among the patients treated in Mexico City, lasting clinical recovery during the period of observation occurred in only 50 per cent (Table XIV). Most patients showed definite initial clinical improvement following the combined treatment, but many eventually experienced relapses The discrepancy between the results obtained in infections due to Br melitensis and those due to Br abortus is not entirely unexpected in the light of experimental findings in chick embryos In this connection, Magoffin (43a) has demonstrated that Br melitensis is less effectively eradicated from the tissues than Br abortus when these embryos are treated with the combination of streptomycin and sulfadiazine There is also the possibility that many of the apparent relapses were actually reinfections Br. melitensis is transmitted to inhabitants of Mexico City by way of goat's cheese as well as other dairy products Patients in Mexico are no less exposed to such foods following treatment than before

The administration of these drugs has varied with respect to dosage and duration. The optimum schedule appears to consist of 0 5 Gm of streptomycin given every 8 hours for 2 weeks and 1 Gm of sulfadiazine every 6 hours for 2 weeks The total dose of streptomycin is 21 Gm when administered in this fashion An initial dosage of 4 Gms of sulfadiazine is given at the time streptomycin therapy is started, following which sulfadiazine is given as already described Schedules involving larger daily amounts of streptomycin are undesirable because of the danger of toxicity, especially eighth nerve damage. Shorter courses of streptomycin appear to be

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At the present time, uninterrupted recovery has been observed in 22 of the 24 treated cases over periods ranging from 1 month to 3 months after treatment. Repeated blood cultures taken after treatment have all been sterile in the patients who did not experience clinical relapse. In 2 patients, fever and bacteremia returned One of these had been critically ill for 6½ months, with complications of splenomegaly, purpura hemorrhagica, and severe anemia, and was not expected to survive. Her recovery after treatment was dramatic. After receiving aureomycin for 5 days the fever, purpura, and splenomegaly subsided. Bacteremia and low-grade fever recurred 3 weeks after treatment was discontinued, but her general condition remained good

Most patients in this series received approximately 18 Gm. of aureomycin over a period of 10 days. A sudden high elevation of temperature was observed repeatedly among the first patients treated. In some cases this elevation was accompanied by symptoms of peripheral vascular collapse, and presented a picture resembling the Herxheimer phenomenon. In order to avoid this reaction, small initial doses were prescribed and increased gradually until the fourth day, when 2 Gm. were given. There were no other important side effects Mild gastrointestinal symptoms, consisting of nausea, *vomiting, and diarrhea,* were present *but transitory.*

Aureomycin promises to qualify in many respects as an ideal agent for treating human brucellosis. It is effective, produces no significant toxic reactions, and can be administered orally. The effectiveness of aureomycin in infections due to Br. suis has not yet been established, but excellent results were observed after the use of this drug in the treatment of 16 consecutive patients with bacteriologically proved brucellosis due to Br. abortus in Minnesota (5a). Among the new antibiotics, chloromycetin (chloramphenicol) has received limited trial, and was reported by Woodward (76) to be of definite value in the treatment of 8 cases of brucellosis.

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Advances in the Neuromuscular Disorders

DONALD MCEACHERN AND REUBEN RABINOVITCH

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Introduction

Efforts of biochemists, biophysicists, and physiologists have illuminated many of the mysteries of normal muscle at work and at rest. Nevertheless, a portion of humanity has continued to drag weak and wasted limbs from one hospital to another in search of aid

Textbooks have grouped together a series of syndromes under such titles as Myopathies, Muscle Diseases, Neuromuscular Diseases. Excellent clinical descriptions remain, some of which are more than a century old Little has been achieved in the past, however, in explaining the morbid processes, other than to distinguish whether the muscle or the central or peripheral nervous system was primarily involved. The present trend is to probe the causes and mechanisms of these conditions, and many advances have been made in the past twenty years. It is along these lines that hope lies for the afflicted

The term "Neuromuscular disorder" includes the conditions which affect, in a systematic way, the lower motor neuron, the neuromyal junction, or the muscle fiber. We group these together not upon anatomic grounds, but because disturbance of one part of the lower motor neuron is apt to involve the other parts. Furthermore, their chemical processes and their vulnerability to noxae are often similar Diseases quite different in causation may produce similar disturbances of function (e g , pain, muscle spasm, fasciculation, atrophy). These often require skilled interpretation by specialized methods Future advances in our understanding and treatment of these conditions must be based upon a knowledge of the physiology and chemistry of the lower motor neuron

Some of the neuromuscular disorders seem to affect only one part of the lower motor neuron, e g , muscle, in muscular dystrophy.

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Some of the neuromuscular disorders seem to affect only one part of the lower motor neuron, e g., muscle, in muscular dystrophy,

Others involve the entire lower motor neuron. In some instances, we may assume out of ignorance that only one part of the unit is affected. We now know, for instance, that poliomyelitis actively involves the whole motor neuron and not alone the anterior horn cell.

The term "neuromuscular disorder," therefore, covers all conditions which affect the lower motor neuron in a systematic way, but it does not include many conditions which accidentally involve the lower motor neuron (e.g., syringomyelia, nerve compression).

No attempt is made in this review to cover the literature, but enough references are given to lead the way (59, 66, 69, 138, 155).

Classification

In Figure 1 is sketched our classification of neuromuscular disorders, based on the newer knowledge of these conditions. The disorders are grouped under four heads, depending on whether they affect chiefly (*1*) the anterior horn cell, (*2*) the motor nerve fiber, (*3*) the chemical transmission machinery at the neuromyal junction, and (*4*) the muscle. Some of these conditions may affect several

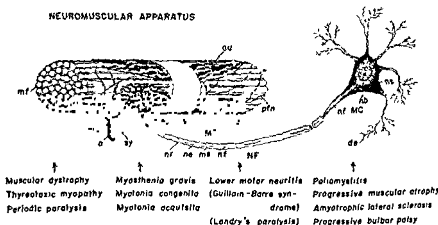


Fig 1 Scheme of the lower motor neuron and examples of disorders affecting various parts Diagrammatic representation of levels of abnormalities in neuromuscular syndromes MC, motor cell, MF, muscle fiber, NF, nerve fiber, a, arteriole, ca, capillary, de, dendrite, mc, muscle cell; mep, motor end plate; mf, myofibril; ms, myelin sheath, ne, neurolemma, nf, neurofibril, nr, node of Ranvier; ns, nucleus; nu, muscle cell nucleus, pfn, perifibrillary network, sa, sarcolemma, sy, sympathetic nerve fiber

parts of the lower motor neuron, and other parts of the nervous system as well.

Physiology of Lower Motor Neuron and Muscle

Nerve Transmission. The lower motor neuron is one of the most specialized structures in the body. It accepts messages, and flashes to muscle the body's signals for posture or motion. It also serves in some nutrient way to preserve the health of the muscle We must learn to look upon its structure not in terms of static histology but in terms of biochemistry Thus, the axis cylinder is not just a wire-like core to the motor nerve fiber. It is, in life, a stream of liquid nutrients constantly flowing from that biochemical factory, the nerve cell, to reactive structures at the periphery. In a sense, it teams up with blood, lymph, and cerebrospinal fluid to form a fourth circulation.

The cell nucleus with its chromatin must be looked upon as a concentration of special enzyme systems and metabolites. Even the Nissl bodies of the cytoplasm are not merely conglomerations of particulate matter, but have been shown in the living cell to alter instantaneously with changes in cell activity. They probably contain specialized enzyme systems necessary to nerve cell function. The process of chromatolysis which results from cell injury must represent a profound change in protoplasmic components and energy relationships in the cell

The nerve cell and its projections have a normal resting metabolism which keeps the conducting machinery tuned up, which manufactures for the future, and which provides energy to maintain local polarization. Stimulation causes immediate depolarization, either due to the electric jolt or to the explosive liberation of a minute amount of acetylcholine (A Ch). Propagation could then be considered to be by electric depolarizations or by a rapid chain reaction involving successive liberations of A Ch along the nerve It is too soon to say which of these mechanisms may be responsible for flow of the action current in nerve (25, 52, 68, 138)

As Aycock and Foley (7) have pointed out, we already know a number of aberrations of nerve cell metabolism resulting from disease These clinical changes must be worked out for many different diseases It is possible, too, that quite dissimilar viruses, toxins, or noxae might attack the lower motor neuron in a similar way by

damaging the same enzyme systems. They might thus produce the same disease picture, e g., Landry's paralysis.

Nerve–Muscle Transmission. Discovery of the chemical factor in neuromuscular transmission has encouraged many to relate certain neuromuscular disorders to abnormality of the transmitting mechanism. Present ideas of normal mediation can be roughly illustrated by Figure 2.

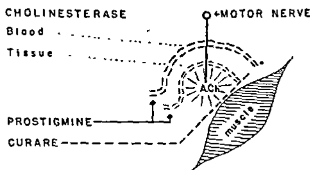


Fig 2. Scheme illustrating present ideas of normal mediation of the transmitting mechanism at the neuromyal junction. Prostigmine is the alternate name for neostigmine

On stimulation of the motor nerve fiber, acetylcholine is exploded in minute amounts at the neuromyal junction or in immediate proximity to the surface of the muscle fiber. This either causes contraction or so conditions the fiber that it responds normally to the electric impulse. The enzyme, cholinesterase, present both in muscle tissue and in the circulating blood, rapidly breaks down any excess A.Ch., thus preventing its spread to adjacent muscle fibers or into the general circulation and limiting its effect, therefore, to the desired point of stimulation Eserine or its analogue, neostigmine, has an affinity for cholinesterase They bind it and "take it out of the play," thus allowing a greater concentration of A.Ch to exert its effect These anticholinesterase drugs therefore potentiate and prolong the muscular stimulation by A.Ch.

Curare and similar drugs have no effect upon the production of A Ch, and they may not alter the activity of cholinesterase, but in some way they reduce the response of the motor end-plate or the muscle fibers to nerve stimulation (38) If curarization is not too heavy, neostigmine will overcome the defect by mobilizing more

A.Ch. A limit to this is reached, however, when all the cholinesterase has been bound and all the available A Ch is in action A larger dose of curare can then not be surmounted by any dose of neostigmine. This scheme may be an oversimplification, but it serves as a working basis.

Muscular Contraction. Much work has been done since the early days of energy metabolism The glycogen–lactic acid cycle, once the be-all and end-all of muscle energy and contraction, is now known to be but one of the alternative fuelling systems. The muscle fiber is a prototype of living organisms; all it does is to contract and relax. This involves the properties of a fibrous protein with immediate energy supplied by dephosphorylization of adenosinetriphosphate (ATP.). The omnipresent muscle protein, myosin, apparently acts as an enzyme, splitting phosphate off the ATP. Such proteins are therefore not just potatoes in the bunker but active agents All protoplasm may be a concentration of enzymes Myosin seems to be contaminated by, or to have as a mate, another protein called actin The two together form the functional fibrous protein of muscle—actomyosin This substance, in the presence of ATP. and potassium, rapidly undergoes extreme shrinkage which resembles muscular contraction. The brilliant work of Szent-Györgyi and of others (171, 155) points a finger to the future. Muscle protein configuration is being studied (53) and the electron microscope (76) may well provide answers for many of these puzzling questions

Pathophysiology of Lower Motor Neuron and Muscle

Spasm Although muscle spasm is one of the most important symptoms in medicine its mechanism is not well understood, indeed, there may be several mechanisms It may occur in all degrees, from the moderate tightness of the suboccipital muscles in the patient with "tension" headache to the violent back spasm in meningitis It may involve large groups of muscles, or even individual muscles or parts of a muscle, as in poliomyelitis It is often demonstrable only when the muscle is stretched Much pain that has hitherto been interpreted as referred pain may well be explained in the future by spasm of muscles deep or remote from the lesion This would explain, too, the pain-relieving effect of heat, which has long been known but little understood Heat tends to relax muscle spasm.

It is necessary to consider several possible elements in the mechanism of spasm. Reflex spasm mediated through the spinal arc can occur, for example, when a wounded muscle is stretched and nearby ones respond in protective spasm. Widespread and irradiating spasm may arise from irritation of a nerve root, as for instance the rigidity of paraspinal muscles and the spasm of hamstrings on stretching—which may occur when a protruded intervertebral disk affects a single nerve root. Spasm may also be due to repetitive volleys from irritated anterior horn cells, as in strychnine poisoning, but whether this operates much in disease is unknown One element in the spasm in poliomyelitis would appear to be due to irritation of anterior horn cells or internuncial neurons in the spinal cord Finally, spasm can be a peripheral affair due to involvement of the neuromyal junctions, as with tetanus (78).

It is not always clear whether spasm is a beneficial process or not The splinting of an injured joint by muscle spasm might be looked upon as one of nature's safeguards, whereas the spasm of tetanus might not In any event, it may be said that the intensity and extent of muscle spasm often seems greater than is necessary or desirable, especially when the patient is at rest, and thus the doctor often takes measures to relieve the spasm.

Heat and massage are the old stand-bys, but more recently certain drugs have been under experimental trial. Curare has received much publicity in this regard. Theoretically, it might be expected to be effective when spasm is due to involvement at the neuromyal junction, since that is where the drug acts. It could, however, be effective in spasm due to reflex or central excitation, if it blocked such abnormal trains of impulses without blocking normal action currents sufficiently to impair voluntary movement. This selective action is claimed for the drug (158) The fleeting effect of an injection of curare does not offer much encouragement for persistent relief of spasm Suspension of the drug in oil gives more lasting action. Schlesinger (158) gives evidence of marked relief of spasm in traumatic and orthopedic states by parenteral injection of 3 per cent tubocurarine in 4 8 per cent wax in peanut oil 1 cc of the suspension contains 175 units of standard curare. Doses ranged from 0 4 to 2 5 cc. Duration of effect was from 24 to 168 hours. Results in poliomyelitis have been contradictory as regards immediate re-

lief of spasm, and unassessable as regards long-term recovery results (140, 149). Our own observations indicate that curare does not inhibit muscle spasm in poliomyelitis in a dose that is safe.

Neostigmine has more support (21, 63, 99, 179) as a relaxant of spasm, and this effect can be observed after an injection (0.5–1 0 mg) or achieved more consistently by giving the drug by mouth (15 mg. every 4 or 6 hours). The drug, of course, potentiates reactions at the neuromyal junction, but it also has an inhibitory action on the spinal cord, as Schweitzer, Stedman, and Wright first showed (160). It is supposed that its beneficial action in poliomyelitis is due to an increased responsiveness of injured internuncial neurons which can then inhibit hyperactive two-neuron reflexes responsible for the painful spasm.

A great opportunity lies ahead for delineation of the pattern of muscle spasm in various diseases With the aid of electromyography, with the additional use of novocaine nerve block or spinal anaesthesia, and with added help from such drugs as curare and neostigmine, it should be possible to pin down the site and mechanism of spasm in various diseases Furthermore, there is the formidable but rewarding task of mapping out with the electromyograph the areas of muscle spasm which result from innumerable lesions of the body tissues or skeleton This would rival in importance and complement the monumental studies on pain reference that have been carried out by Lewis (115) and Kellgren (102)

Cramp Muscle cramp consists of the violent, involuntary, and painful contraction of a muscle or a portion of it Most people have experienced it after unaccustomed exercise or in the form of a "stitch" (intercostal) It may, however, be due to one or another of a number of conditions which embarrass the muscle's metabolism, such as relative anoxia (either general or due to local vascular disease), hypocalcemia, or thiamine deficiency In these instances cramp usually follows upon exercise.

Cramps which develop at rest, or during the first use of the muscles upon awakening, commonly occur, along with fasciculations, in patients with progressive muscular atrophy It would seem either that the chemical transmission machinery at the neuromyal junction was overactive, or that the junction had become sensitized to acetylcholine (141) Night cramps may plague normal old people and wake them from sleep It is important to remember that quinine in

doses of 5 Gr. thrice daily will often abolish these cramps, perhaps due to the curariform effect of the drug.

The electromyogram, as one might expect, shows an intense discharge of a nervous type, although undoubtedly of primarily muscular origin. Once set up, it tends to involve the whole muscle, or muscle group, becoming a reflex spasm induced from the muscle itself (45). Relaxation is assisted by tensing antagonists, as in the familiar gesture of leaping from bed and standing on the toes or heels to overcome a calf cramp

Cramp is popularly believed to be the cause of many accidental drownings. This is, in our opinion, an "Old Wives' Tale" It is difficult to conceive of a cramp so general and so violent that it would double a man up and sink him We suspect that most of these victims have had an epileptiform seizure; 3 of our epileptic patients have been drowned because of "cramps" while swimming.

Denervation Under the title *A Law of Denervation* (27) the late great Professor Walter Cannon marshalled a variety of facts. Tissues, when deprived of their nerve supply, develop after several weeks an acute sensitiveness (10–1,000-fold) to the chemical transmitter which formerly served them, and to other agents too. Thus, blood vessels, after section of the adrenergic sympathetic nerve supply, contract with doses of adrenalin many times less than those that stimulate normal blood vessels Similarly, a denervated striate muscle contracts with infinitesimal doses of its chemical transmitter —acetylcholine. This explains the Sherrington phenomenon of contraction of denervated limb muscles on antidromic stimulation of the posterior nerve root Other cholinergic fibers (vasodilator) release enough A Ch. peripherally to stimulate the sensitized muscle This response does not ordinarily occur because, normally, skeletal muscle is not excited by the concentration of A.Ch which is set free from the vascular muscles The Vulpian phenomenon of slow contraction of the denervated tongue upon stimulation of the chorda tympani is another example Bender (13) has shown that spasm of denervated facial muscles may occur in the monkey with fright or surprise. This is apparently due to liberation of minute amounts of A.Ch. into the blood stream We have studied a case of longstanding Bell's palsy in which unilateral facial spasm occurred during sudden emotional alert

Dilatation of the cat's pupil, sensitized by previous section of

its sympathetic nerve supply, is a common assay method for minute amounts of adrenalin. These and many other examples of denervation are dealt with by Cannon

The process of sensitization occurs not only in muscles deprived of their motor nerve supply, or in structures cut off from postganglionic autonomic fibers, but at higher levels as well. Thus, anterior horn cells become sensitized after section of the pyramidal (upper motor neuron) fibers which lead to them. In this case, the muscle becomes moderately sensitized too, but not nearly so much as the anterior horn cell, and very much less than if its own motor nerve fiber (lower motor neuron) had been severed

In like manner, the postganglionic units of the autonomic nervous system become sensitized after section of the preganglionic fibers. It has even been suggested that Jacksonian epilepsy might be due to sensitization of cortical neurons which have been partially deprived of nervous connections by disease or injury (27). Cannon stated his "law" as follows: "When in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effect being maximal in the part directly denervated."

The above concept has real practical applications in the field of neuromuscular disease It probably explains many instances of cramp, spasm, and fasciculation It may, in the future, explain convulsive and other phenomena stemming from the central nervous system

Fibrillation. This phenomenon was first described by Schiff in 1851, and later by Langley and Kato (112) It consists of continuously repeated, fine contractions of muscle fibrils It is seen characteristically in denervated muscle and begins a few days after nerve section and continues indefinitely thereafter. This process can be observed by the physiologist in exposed muscle, where it appears as a barely perceptible rippling. The term is a misnomer, however, when applied to the grosser twitchings of sheets or fasciculi of muscle apparent to the clinician in certain neuromuscular diseases The latter is more properly termed *fasciculation* Fibrillation occurs, of course, in man, but is seldom discernible through the human integument, although it can be readily recognized in electromyograms recorded from a needle placed in the muscle belly. By this

refined method partial states of denervation may be recognized by the neurophysiologist, and indeed the process of recovery can be followed, since fibrillation disappears with regrowth of the motor nerve fiber and reestablishment of its normal connections (46, 62)

Fasciculation. This grosser type of muscular twitching seen by the clinician is due to the sudden contraction of a number of muscle fibrils, perhaps the simultaneous contraction of all those served by one or more motor nerve fibers. One fiber may innervate 100 to 150 muscle fibrils—the whole complex being called a motor unit Fasciculation gives quite a different picture from fibrillation on the electromyogram.

Although fasciculation is usually seen in conditions involving damage to anterior horn cells (progressive muscular atrophy) it can no longer be looked upon as the last death rattle of the anterior horn cell which is sending down abnormal volleys to its motor unit To begin with, the phenomenon is a peripheral one, and complete blockage of the motor nerve with novocaine fails to affect the fasciculation in the slightest (141). The phenomenon may often be due to sickness of the anterior horn cell, but it is almost certainly due to a coordinated explosion at the periphery, through an axon reflex, arising at the neuromyal junctions of a motor unit Denny-Brown and Pennybacker (46) and Ford (62) have indeed shown that active fasciculation may occur in a degenerating motor unit which is capable of a normal, *willed contraction on the part of the patient.* The anterior horn cell and its lower pathways must therefore be relatively intact in such a situation It is well known that when complete denervation occurs in progressive muscular atrophy, and paralysis and muscular wasting set in, fasciculation disappears No doubt electromyographic records would still show active fibrillation in these seemingly inactive muscles, at least until fibrosis has occurred

Furthermore, fasciculation can occur in the complete absence of motor neuron degeneration We have examined several patients (two of them physicians) with widespread muscular fasciculation which has continued for years with no evidence of muscle atrophy or neurologic disease Patients with thyrotoxic myopathy may exhibit severe muscular weakness, wasting, and fasciculation, and the whole process clears up following thyroidectomy An ordinary dose of neostigmine administered to a normal animal or human can cause

widespread muscular fasciculation. It is of interest that this can be abolished at once in animals (141) and humans (96) with a dose of curare which causes no evident muscular weakness This might be expected from our knowledge of the peripheral, neuromyal action of both drugs

Curare is capable also of stopping fasciculation temporarily in patients with progressive muscular atrophy, but this is not practical therapy. We have tried quinine by mouth, but it is not effective in therapeutic doses

Many patients who have lost weight and are thin and depleted from a variety of constitutional diseases exhibit a heightened neuromuscular excitability which is evidenced by fasciculation or by increased myotactic irritability when the muscle is stretched or percussed (contraction fasciculation, myokymia) Attempts have been made (46) on clinical and electrographic grounds to differentiate these phenomena It is doubtful whether such a division is profitable, since they often occur together and do not necessarily indicate motor neuron disease. Masland and Wigton (120) have shown by electromyography that outbursts of electric activity originating at the neuromyal junction are conducted antidromically up the motor nerve fiber during fasciculation. What might happen to the anterior horn cell under such bombardment over a long period of time no one knows.

Atrophy Atrophy of muscle occurs when it has been denervated either mechanically or by disease The muscle shrinks, tone and tendon reflexes disappear, electric reactions are altered, and fine, continuous fibrillation sets in

Atrophy of muscle is therefore very common In certain heredofamilial and degenerative diseases which affect anterior horn cells in addition to pyramidal or other long tracts, secondary degeneration of the muscle fibers occurs due to denervation In this group belong the spinal muscular atrophies of the Werdnig-Hoffman type, hereditary spastic paraplegia, hereditary ataxia with muscular atrophy, and the Charcot-Marie-Tooth type of peroneal muscular atrophy These conditions are not discussed in detail here

Characteristics common to this group are (*1*) a positive family history, (*2*) onset of progressive weakness and atrophy fairly early in life, (*3*) the paucity of symptoms and signs save those due to

lower motor neuron lesions, and (4) ignorance of the cause of the disorders. The work of Julia Bell (11) is probably the best available reference on this subject. She demonstrates, by means of pedigrees culled from the literature and from her own cases, the almost monotonous regularity with which these forms of muscular atrophy reappear in one or several members of a family, generation after generation. The hereditary character of these conditions is apt to plunge us into hopeless pessimism. This is not warranted. The conditions are each probably due to lack of a specific gene supplying some essential enzyme system which might be supplied artificially, if known. After all, familial periodic paralysis is but an inborn error of potassium metabolism.

There is no reason to believe that the muscular atrophy encountered with neuromuscular disease is any different from that due to mechanical denervation. The process may, of course, be slower, and the neuron may be sick for a long time before its life is extinguished Profound muscular atrophy may also result from disuse

The most recent attempts to simulate atrophy resulting from a process which is either primarily located in the lower motor neuron or in the muscle fibers are fully reviewed by Hoagland (85). In order to study both disuse and motor denervation effects on muscles, experimental animals have been subjected to either denervation, tenotomy, or immobilization by cast The alterations of muscle chemistry described by various authors have been summarized in chart form (Table I). It is believed that the changes in the chemical constituents of muscle are due to progressive alteration in the relative quantities of muscle cell and connective tissue phases. Thus, lactic acid formation, glutathione content, and collagen concentration are increased in either denervated or inactivated muscles. The oxygen uptake is either increased or decreased, depending upon the species and procedure employed The total protein concentration, the precipitable myosin, creatin content, glycogen content, phospholipids, glucose, lactic acid, oxygen and carbon dioxide, succino-dehydrogenase activity, weight, birefringence, and contractile power have most often been reported as decreased There is no clear-cut biochemical difference between muscles which have atrophied due to denervation and those suffering disuse atrophy.

The course of muscle weight loss in atrophy of the gastrocnemius

muscles of rats following lower motor neuron lesion, upper motor neuron lesion, and skeletal fixation, is shown by Solandt *et al.* (167) to be the same for the first 10 days. From then on the course of the atrophy differs. That due to a lower motor neuron lesion or to skeletal fixation progresses until the muscle fibers are destroyed, while that due to an upper motor neuron lesion shows improvement, sometimes with a return to normal. Admitting that the genesis of denervation atrophy remains obscure, Solandt and co-workers nevertheless agree with Lazere, Thomson, and Hines (113) that it is not due to overwork resulting from fibrillary activity, as believed by

TABLE I

*Experimental Muscular Atrophy following Denervation, Tenotomy, or Immobilization**

Lactic acid formation ↗	Succinodehydrogenase activity ↘
Glutathione content ↗	↗
Collagen concentration ↗	*Blood constituents*
Oxygen uptake ↗	↘
Creatine ↘	(glucose, lactic acid, O_2, CO_2)
Glycogen ↘	Weight ↘
↗	Birefringence ↘
Phospholipids →	Contractile power ↘
↘	Total protein concentration ↘
	Precipitable myosin ↘

* Summary of reports in the literature on biochemical analyses of muscle tissue in animals where atrophy was obtained by denervation, tenotomy, or immobilization of a limb. Several authors (85) have found an increase (↗), decrease (↘), or unchanged state (→) of substances or of properties listed in this table

Langley and Kato (112). Some benefit resulted from muscular activity induced either mechanically or by periodic electric stimulation Best results were obtained with the 25-cycle alternating current (166). The improvement is perhaps due to the provoked increase in blood supply Induced activity may reproduce the propulsive effect of normal muscular contraction on a stagnant circulation, thus facilitating muscle metabolism pending its reinnervation Increase in strength, observed during recovery following partial denervation, may be due to hypertrophy of residual motor units or to further ramification of nerve fibers from adjacent intact segments.

Kenny Treatment. Although muscle spasm and several of her

other concepts had been observed and forgotten prior to Sister Kenny's tempestuous arrival (105) the credit goes to her for revolutionizing our conception and treatment of poliomyelitis It is useless for doctors to deny this, although there have been numerous rather pathetic attempts to do so in papers and official reports. The fact that her attempts to construct a neurophysiologic rationale were naïve is of little consequence. Her observations are the important thing

Muscle spasm in poliomyelitis is complex and probably contains reflex, cord, and neuromyal elements. There is no doubt, however, that it can be relieved temporarily and quickly by the use of moist heat, and it is probable that consistent packing shortens the total duration of spasm. Spasm due to other conditions, from lower motor neuronitis (126) to protruded intervertebral disk, is also benefited by hot packs No attempt will be made here to assess the value of hot packing and the other therapeutic measures which comprise the Kenny method (34,109). Sufficient to say that the method, with various modifications, is employed in most hospitals today.

Best results often depend upon very frequent change of hot packs, even every 10 minutes. This has led to the development of ingenious apparatus (e g , the Vollrath Polio-Pak Heater*) for the preparation of hot packs at the bedside One of these is pictured in Figure 3

Electromyographic records of the effects of hot packing on muscle spasm have been presented by Watkins and Brazier (179)

Nutritional Muscular Dystrophy. Following Evans and Bishop's (57) original discovery of vitamin E in 1922, Einarson and Ringsted (54), in 1938, were the first to study systematically the neuromuscular degeneration and paralysis which occurs in suckling rats when the mother is maintained on a diet deficient in vitamin E They produced a similar condition in adult rats on a deficient diet prolonged over many months Wheat germ (rich in tocopherols, i e , vitamin E) protected against this Since that time numerous workers have succeeded in producing a kind of muscular dystrophy in a variety of animals, including rabbits, guinea pigs, ducklings, and rats (42, 72, 116, 142, 144) This was achieved by feeding diets deficient in vitamin E, and was prevented or cured by the addition of wheat germ or of the synthetic vitamin, alphatocopherol Dys-

* The Vollrath Co , Sheboygan, Wisconsin

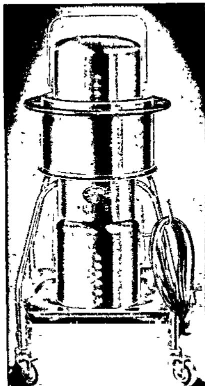


Fig 3 Mobile unit for preparation of hot packs at the bedside (courtesy The Vollrath Co)

trophy has also been produced in dogs with biliary fistula while fed a normal diet Here the diversion of bile resulted in malabsorption of the fat-soluble vitamin E (22) In experimental muscular dystrophy, creatinuria occurs and can be used as a measure of the condition Dystrophic muscle shows a greatly increased oxygen consumption *in vitro*, which returns to normal by addition of alpha-tocopherol (91) either in the diet of the animal or *in vitro* (92, 101) The biochemical changes which occur in the dystrophic muscle according to various authors (71, 107, 135–137) are summarized in Table II

From the beginning, attempts were made by clinicians to discover the neuromuscular disease in man which might be the counter-

part of nutritional muscular dystrophy in animals. Therapeutic experiments were undertaken, but some of the early reports of improvement of muscular dystrophy (15, 170) and of amyotrophic lateral sclerosis (181) were overexuberant and uncritical. Furthermore, in some reports it is not clear whether wheat germ, wheat germ oil or one of its fractions, or alphatocopherol was used.

Numerous workers have now published negative results with some of these agents in muscle dystrophy and amyotrophic lateral sclerosis (49, 61, 175).

TABLE II

*Muscle Changes and Metabolic Disturbances in Experimental Animals with Vitamin E Deficiency**

(71, 107, 135–137)

Muscle Analyses	*Metabolic Changes*
Water, calcium, and sodium chloride content ↗	Glycemic curve ↗
Total fat ↗	Glucose, lactic acid, total acid soluble phosphorus (fasting blood) → ↗
Lipoid phosphorus ↗	
Oxygen consumption ↗	Creatin, creatinine → ↘
Succinoxidase activity ↗	
	Cholesterolemia ↗

* Biochemical analyses of tissues in animals in which muscular dystrophies were obtained following dietary deficiencies Substances or properties listed here have shown increase (↗), decrease (↘) or unchanged states (→).

We present our own experience (Table III), since it seems to be the most extensive recorded and covers 151 patients with various neuromuscular diseases, some of whom have been under study since 1936 The results will be reported in detail elsewhere (148).

Wheat germ oil,* Milhorat fraction† (128) of wheat germ oil, and synthetic alphatocopherol have been used alone and in combination, and in doses as high as 300 mg daily when computed in terms of alphatocopherol, although the average was less than this, approximating 60 mg. daily. Bile salts were given in some instances to promote absorption. As can be seen in Table III, of a total of 107

* ... Ltd , St.
... lene dichlo-
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patients treated with wheat germ oil, no beneficial effects were observed in amyotrophic lateral sclerosis, myasthenia gravis, disseminated sclerosis and a number of other conditions.

There were 13 patients in the series who, in our judgment, showed sufficiently striking improvement to warrant the conclusion that

TABLE III

Patients Investigated in the Neuromuscular Disease Clinic

Diagnosis	Cases investigated	Cases treated with wheat germ oil	Number improved
Conditions improved			
Pseudoprogressive muscular atrophy	2	2	2
Progressive muscular dystrophy	29	25	5
Menopausal muscular dystrophy	7	5	3
Dermatomyositis	3	3	3
Conditions unimproved			
Progressive muscular atrophy	16	11	
Amyotrophic lateral sclerosis	20	14	
Progressive bulbar palsy	1	1	
Motor neuronitis	3	3	
Myasthenia gravis	24	11	
Myotonia congenita	2	2	
Myotonia atrophica	2	0	
Peroneal muscular atrophy	1	1	
Thyrotoxic myopathy	1	0	
Dystonia musculorum deformans	1	1	
Friedreich's ataxia	2	2	
Obliterative arterial disease	3	1	
Disseminated sclerosis	9	9	
Unclassified myopathies	25	16	
Total	*151*	*107*	

treatment was responsible Improvement of symptoms was apparent in 2 cases of pseudoprogressive muscular atrophy and in 5 cases of progressive muscular dystrophy (3 pseudohypertrophic type and 2 adult type) The 2 pseudoprogressive muscular atrophy patients were adult women who had suffered motor neuron disease in early

life with a later exacerbation at the involutional period These are difficult to classify. Relapse upon discontinuing treatment and improvement when it was resumed were noted in 3 patients; 3 other patients, all of whom improved, were labeled as cases of dermatomyositis These exhibited a rubbery kind of dystrophic process in the muscle, along with scleroderma-like changes in the skin Milhorat *et al.* (130, 131) have described 2 cases of dermatomyositis improved by fresh wheat germ. Our remaining 5 patients, women at about the climacteric age, exhibited a proximal type of muscle weakness involving mostly shoulder and pelvic girdle muscles; 3 recovered almost completely. We have called this "menopausal muscular dystrophy," although the term is perhaps not a good one From our experience it is impossible to say whether alphatocopherol or other factors in wheat germ oil were responsible for the improvement, since wheat germ oil, sometimes supplemented by alphatocopherol, was used in all these successful instances

Mention should be made of the work of Milhorat and Bartels (128) who claimed that a sort of condensation of alphatocopherol and inositol occurs during normal digestion to produce the antidystrophic factor, and that the stomachs of dystrophic patients are deficient in this capacity Later, a special fraction (128) was prepared by extraction of ether-defatted wheat germ with ethylene dichloride. This was supposed to be 15 times more effective than wheat germ oil An artificial condensation product, a monoether of inositol and tocopherol, was said to be many times more potent in reducing creatinuria in muscular dystrophy cases The above work rests on scant foundation and has not been substantiated Our own therapeutic use of alpha-tocopherol* plus inositol* and of the Milhorat fraction of wheat germ oil has been negative in cases of progressive muscular dystrophy

There are difficulties in establishing a human counterpart to the neuromuscular disorder seen in animals with vitamin E deficiency A similar disease in man might be chronic and might not respond dramatically to treatment Further, one would have to postulate some fault of digestion or absorption of the vitamin, since many of these patients take a normal diet It is of interest that vitamin E is ineffectual when given parenterally to animals with nutritional mus-

* Supplied by Merck and Co, Inc, Rahway, N J

cular dystrophy. Recovery occurs, however, when tocopherol is given by mouth, indicating that the vitamin is conjugated or changed in some way during digestion to form the antidystrophic principle. Finally, there is a regrettable confusion in clinical reports as to whether pure alpha-tocopherol, mixed tocopherols, whole wheat germ, wheat germ oil, or one of its fractions has been used These substances are all lumped together as "vitamin E," whereas some of them contain little of it and others contain a variety of additional substances that might be effective

Wheat germ, so essential to the normal growth of the cereal, is entirely milled off in the making of flour—a situation analogous perhaps to disappearance of vitamin B complex factors with the bran during the polishing of rice. One cannot help but feel that there may be a large and undiscovered territory for the use of wheat germ and its fractions in nutritional and perhaps in hormonal disease.

Hypertonic and Hypotonic Babies. Some babies after birth are rather rigid and have hyperreflexia, others are floppy and quite hypotonic, and tendon reflexes are elicited with difficulty. The babies, within a few weeks or months, assume normal status and develop along normal lines These differences are common knowledge among pediatricians but little is known about the mechanism. An interesting study could be conducted on this matter

"Restless Legs" Under this title Ekbom (55) has recently described a hitherto overlooked disease characterized by paresthesia, pain, and weakness, especially in the lower limbs. The condition is common and may continue for years, in mild or torturing form, without giving rise to any organic disability It is a clinical entity comparable to acroparesthesia or migraine, and is not a symbolic symptom of neurosis, as the inexperienced are apt to label it

Ekbom's cases fall into two main groups those with paresthesias alone or predominant, and those with pain alone or predominant He studied 34 severe cases and 120 mild cases of the paresthetic form and 15 cases of the painful form. There is no distinct borderline between the two

In the *paresthetic form*, the paresthesias are situated inside the lower legs but generally not in the feet, sometimes in the thighs also, rarely in the arms They are usually referred to the muscles,

sometimes to the bones, never to the skin. They consist of highly disagreeable crawling sensations which compel the victims to keep moving their legs or to walk about, which generally gives relief. The sensations usually set in when the legs have been kept still for a while, or especially after retiring for the night In severe cases they may keep up for hours and greatly disturb the patient's sleep. In about two-thirds of the cases, the legs tire quickly or feel weak or as if they are about to give way at the knees Coldness of the feet is a common complaint, too. Upon examination, Ekbom found only the motor unrest. Spontaneous remissions were not uncommon in his cases, and the condition sometimes made its appearance or became worse during the last half of pregnancy. Good therapeutic results were ascribed to doryl (carbaminoylcholine chloride) 0 002 Gm. 4 to 6 times daily by mouth, or priscoline (benzazoline hydrochloride) 0 025 Gm. 3 times daily by mouth.

In the *painful form,* the pain consists of mild to moderate aching and is situated in the same places as paresthesia in the foregoing cases. It is usually worst when the patient is resting, and forces him to move his legs The pain may be quite irregular in behavior Crawling sensations, tiredness of the legs, and cold feet may also occur in this form. The crawling sensations are said by Ekbom to be relieved by doryl and priscol, but not the pain.

The mechanism of restless legs is not known, but there are obvious suggestions that it may be a neuromuscular disorder. In one of our cases the condition was associated with painful "rest" cramps in the leg muscles at night and involuntary jerkings of the large muscles similar to but more extensive than fasciculations The cramps were relieved by 5 grains of quinine thrice daily, by mouth.

The condition sometimes runs in families and is popularly termed "leg jitters" Allison (2) found that nitroglycerine, 1/100 grain, dissolved under the tongue, gave relief.

Tetany. This state of heightened neuronal and neuromuscular excitability is common to many metabolic disorders Brain cells may be affected (convulsions), spinal reflex arcs (hyperreflexia), and the neuromuscular apparatus as well (cramps and twitchings) The causes are many. parathyroid deficiency, vitamin D deficiency, lack or loss of calcium in the diet, alkalosis from various causes includ-

ing the blow-off of CO_2 during hyperventilation. In general, it seems that reduction of ionized calcium in the tissue cells is the main common denominator (14). This, however, may not explain all instances. Calcium deficiency and alkalosis seem to differ.

Cramps and spasmophilia are the main events in the neuromuscular field and they lead to characteristic carpopedal spasm, positive Chvostek and Trousseau signs, laryngeal spasm, and other manifestations. Analysis of the electromyographic abnormality has been made. According to Masland (119), it is akin to that found in botulism.

Patients with tetany due to hypocalcemia are very sensitive to epinephrine (82) Intra-arterial injection of the drug in such cases evokes immediate local tetany and prolonged local vasoconstriction This hypersensitivity to epinephrine does not occur in hyperventilation tetany The potassium-calcium ratio and the effect of epinephrine on the migration of potassium ions are probably related to the development of this sensitivity Tetany highlights the wide range of physicochemical abnormalities that can disturb the neuromuscular system.

West (184) has studied parathyroid tetany in animals His results indicate that the disorder affects all motor neuron structures from top to bottom

Methods for Study of Neuromuscular Disorders

Clinical Tests of Muscle Efficiency It is desirable to employ uniform tests to measure muscular strength and endurance and to evaluate the immediate effect of drugs or the long-term results of therapy. Many methods have been worked out, but we describe below the routine procedures used in our Neuromuscular Disease Clinic

It is recognized that all tests of strength or fatigability are at best relative and depend upon a variety of factors, including not only organic disease or dysfunction of tissues, but also rate of work, effect of training, effect of psychologic factors on cooperation during tests, and uniformity of methods of testing In order to standardize methods of examination the following plan is carried out

The method used in estimating muscle strength is that of grading manual muscle tests as described by Daniels, Williams, and

sometimes to the bones, never to the skin. They consist of highly disagreeable crawling sensations which compel the victims to keep moving their legs or to walk about, which generally gives relief The sensations usually set in when the legs have been kept still for a while, or especially after retiring for the night. In severe cases they may keep up for hours and greatly disturb the patient's sleep In about two-thirds of the cases, the legs tire quickly or feel weak or as if they are about to give way at the knees. Coldness of the feet is a common complaint, too. Upon examination, Ekbom found only the motor unrest. Spontaneous remissions were not uncommon in his cases, and the condition sometimes made its appearance or became worse during the last half of pregnancy Good therapeutic results were ascribed to doryl (carbaminoylcholine chloride) 0 002 Gm. 4 to 6 times daily by mouth, or priscoline (benzazoline hydrochloride) 0 025 Gm. 3 times daily by mouth

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Tetany This state of heightened neuronal and neuromuscular excitability is common to many metabolic disorders. Brain cells may be affected (convulsions), spinal reflex arcs (hyperreflexia), and the neuromuscular apparatus as well (cramps and twitchings) The causes are many parathyroid deficiency, vitamin D deficiency, lack or loss of calcium in the diet, alkalosis from various causes includ-

LEFT									RIGHT
					Examiner's Initials					
					Date					
SCAPULA					Abductor — Serratus anterior					SCAPULA
					Adductor — middle trapezius					
					Adductors — Rhomboids					
					Elevators					
					Depressor					
SHOULDER					Flexors					SHOULDER
					Extensors					
					Abductors					
					Horizontal Abductor					
					Horizontal Adductor					
					External rotators					
					Internal rotators					
ELBOW					Flexors					ELBOW
					Extensors					
FOREARM					Supinators					FOREARM
					Pronators					
WRIST					Flexor — radial deviation					WRIST
					Flexor — ulnar deviation					
					Extensors — radial deviation					
					Extensor — ulnar deviation					
FINGERS					Flexors — metacarpophalangeal					FINGERS
					Extensors — metacarpophalangeal					
					Flexor — proximal interphalangeal					
					Flexor — distal interphalangeal					
					Abductors					
					Adductors					
					Opponens — 5th finger					
THUMB					Opponens					THUMB
					Flexor — metacarpophalangeal					
					Extensor — metacarpophalangeal					
					Flexor — interphalangeal					
					Extensor — interphalangeal					
					Abductors					
					Adductor					
					MEASUREMENTS					
CHEST					Inspiration					CHEST
					Expiration					
ABDOMEN					Umbilicus to Ant. Sup. Spine					ABDOMEN
LOWER EXTREMITY					Circumference — mid calf					LOWER EXTREMITY
					Circumference — mid thigh					
					Ant. Sup. spine to int. malleolus					
					Umbilicus to internal malleolus					

Cannot walk Date________ Walks with crutches Date________

Stands Date________ Walks with canes Date________

Walks with braces Date________ Walks unaided Date________

Walks with corset Date________ Climbs stairs Date________

Other Apparatus________

Scoliosis and other deformities________

Supplied by The National Foundation for Infantile Paralysis, Inc., 120 Broadway N. Y. 5, N. Y., Publication No. 60.

Revised March 1946

Reverse side of chart.

Muscle Examination

Patient's Name ________________ Chart No. ________

Date of Birth ________________ Name of Institution ________________

Date of Onset ________________ Attending Physician ________________ M. D

Diagnosis:

LEFT										RIGHT
					Examiner's Initials					
					Date					
NECK					Flexors					NECK
					Extensors					
TRUNK					Flexor					TRUNK
					Extensors — thoracic					
					Extensors — lumbar					
					R ext. obl. / L. int obl } Rotators { L. ext. obl / R int. obl					
					Elevation of pelvis					
HIP					Flexors					HIP
					Extensors					
					Abductor					
					Adductors					
					External Rotators					
					Internal Rotators					
					Sartorius					
					Tensor fasciae latae					
KNEE					Flexor — outer hamstring					KNEE
					Flexors — inner hamstrings					
					Extensors					
ANKLE					Plantar flexors — Gastroc & Soleus					ANKLE
					Plantar flexor — Soleus					
FOOT					Invertor — Anterior tibial					FOOT
					Invertor — Posterior tibial					
					Evertor — Peroneus brevis					
					Evertor — Peroneus longus					
TOES (4 lateral)										TOES (4 lateral)
HALLUX					Flexor — metatarsophalangeal					HALLUX
					Flexor — interphalangeal					
					Extensor — interphalangeal					

Additional Data

Face ________________

Speech ________________

Swallowing ________________

Diaphragm ________________

Intercostals ________________

KEY

[illegible]

Fig 4 Chart for muscle examination

LEFT									RIGHT
					Examiner's Initials					
					Date					
SCAPULA					Abductor — Serratus anterior					SCAPULA
					Adductor — middle trapezius					
					Adductors — Rhomboids					
					Elevators					
					Depressor					
SHOULDER					Flexors					SHOULDER
					Extensors					
					Abductors					
					Horizontal Abductor					
					Horizontal Adductor					
					External rotators					
					Internal rotators					
ELBOW					Flexors					ELBOW
					Extensors					
[illegible]					Supinators					[illegible]
[illegible]					[illegible]					[illegible]
					Adductors					
					Opponens — 5th finger					
					Opponens					
[illegible]					[illegible]					THUMB
					MEASUREMENTS					
[illegible]					[illegible]					[illegible]

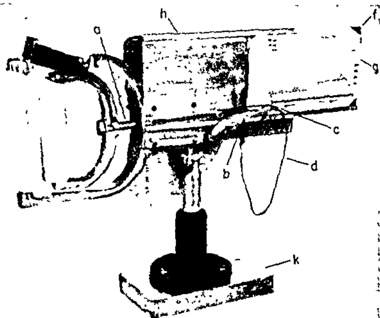
Cannot walk Date________ Walks with crutches Date________
Stands Date________ Walks with canes Date________
Walks with braces Date________ Walks unaided Date________
Walks with corset Date________ Climbs stairs Date________
Other Apparatus________

Scoliosis and other deformities________

Supplied by The National Foundation for Infantile Paralysis, Inc., 120 Broadway, N. Y. 5, N. Y., Publication No. 44.

Revised March 1946

Reverse side of chart.



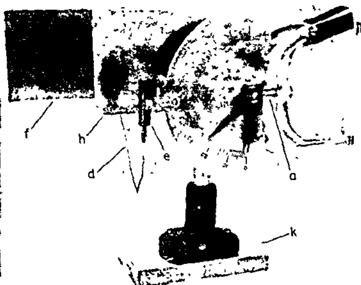
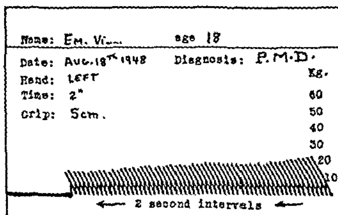
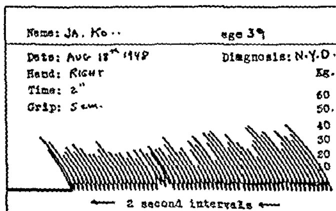


Fig 5 Modified Smedley hand dynamometer The piston (a) displacements are translated to a lever (b) carrying a short piece of capillary tubing (c) ink fed (d) from a reservoir (e) An interchangeable card holder (f) carrying standardized scale cards (g) mounted in a flanged frame (h) was made to move by means of a ratchet system The heavy metallic base (k) eliminates the need for fixation, thus making the apparatus portable The test is standardized at 60 "squeezes" at 2 second intervals



A



B

FIG 6 Two sample graphs obtained on the modified Smedley hand dynamometer Maximum hand grip strength, fatigability curve and psychomotor behavior are recorded A, Initial strength, 28 Kg , fatigability curve after 60 squeezes, 20 Kg , performance from one squeeze to the next, fairly uniform B, Initial strength, 45 Kg , final strength, 35 Kg , performance from one squeeze to the next markedly erratic

Worthingham (39) Records are kept on forms supplied by the National Foundation for Infantile Paralysis (Fig 4, pages 222–223)

The diaphragm, the intercostals, the orbicularis oris, and buccinator muscles are tested by spirometry, both by recording the maximum height to which a column of mercury can be raised by blow-

ing, and the length of time that the mercury level can be sustained at a given height.

The forearm and hand muscles are tested on a Smedley dynamometer. An ink-writer device has been adapted to this dynamometer (Fig. 5) and the resulting graph forms a permanent record Uniform timing is obtained through the use of a metronome set to beat every 2 seconds. The arms and shoulder muscles are tested by weight pulling on a wall-type combination exerciser The thigh and leg muscles are tested on a bicycle ergometer. The written graph obtained on our modified Smedley hand dynamometer permits a fairly critical estimate of the patient's capacity to synchronize squeezing of the hand grip with each click of the metronome, while the fluctuations of the curve obtained by the ink-writer device yield a quite clear pattern of the patient's willingness and concentration Examples of excellent and of poor cooperation are illustrated in Figure 6.

Effect of Drugs. We should think of drugs not as medicaments but as powerful enzyme poisons or as competitors for essential cellular metabolites Neostigmine, eserine, and diisopropyl fluorophosphate (DFP) are strong anticholinesterases and are used in the treatment and investigation of certain neuromuscular conditions Their efficiency does not necessarily parallel their anticholinesterase power

A small injection of neostigmine (0 5 mg.) may bring out a burst of fasciculations in a patient with progressive muscular atrophy where the muscles are on the verge of it Conversely, the patient with myasthenia gravis may absorb huge doses (20 tablets of 15 mg each by mouth) with only an increase in muscular strength and no fasciculation nor visceral phenomena The drug, when given to a normal individual, may cause muscle cramps and fasciculation when given in intermediate dosage These same distinctions obtain locally if the drug is given by intra-arterial injection into the muscles of a limb

Curare, most useful by intravenous injection and with fleeting effect, and also quinine by mouth, have opposite actions to the anticholinesterases Curare in oil or wax is absorbed more slowly but the same difficult differential arises, namely, the therapeutic value balanced against paralytic effects There is promise that a new de-

rivative, *dimethyl-tubocurarine iodide* (Lilly) may provide an answer.

DFP. is a powerful drug that can reduce blood cholinesterase to zero, and also lower that of tissues (81). It improves muscle power to some extent in myasthenia gravis but has yet to be proved a useful therapeutic tool The new drug myanesin (British Drug Houses), developed for possible curariform effects, is a potent one but its main action is more centrally located (168). There are wonderful possibilities in a number of new drugs and variants of old ones, and they must be tested objectively.

Schweitzer *et al* (160), and later Nachmansohn *et al.* (138), gave reasons why various drugs expected to act with similar potency often do not do so Some drugs (e g , eserine) are lipid-soluble and may act in certain locations where the nerve fiber or synaptic structures are clothed by a lipoid sheath Other drugs (neostigmine), being water-soluble, may act only at the postsynaptic membrane where the lipoid sheath is lacking (Fig. 7) This schema could ex-

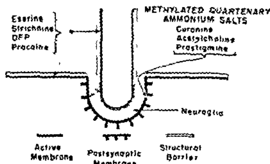


Fig 7 Scheme of the neuromuscular junction (137a) A structural barrier protects nerve and muscle fiber against the action of methylated quaternary ammonium salts These compounds act only on the postsynaptic membrane, which apparently is either less or not at all protected Other compounds, like eserine, DFP, strychnine, and procaine, being able to penetrate the structural barrier, act upon the active membrane of the nerve and muscle fiber, as well as upon synapse

plain many of the seemingly contradictory effects of these drugs.

It would appear that a number of potent drugs (DDT) toxins (botulin) and animal poisons (spider bite) exert their action mainly upon the neuromuscular system

Creatinuria. A high source of energy for contraction of skeletal muscle is the breakdown of phosphocreatine (phosphagen) to its anhydride creatinine. Phosphocreatine acts as a sort of storehouse of energy. It is normally replenished from the creatine ingested in the diet (meat, fish, fowl), and by synthesis from certain amino acids, e g., glycine. Furthermore, as part of the elaborate chemical cycle following muscle contraction, some of the creatinine is almost instantly resynthesized into phosphocreatine. The remaining creatinine is excreted into the urine.

Urinary excretion of creatinine is a normal and regular occurrence. The body's creatine–creatinine balance must however be very delicately adjusted, since an individual's creatinine output is remarkably constant from day to day despite large variations in meat consumption or muscle exercise. The creatinine output is usually about 23 mg. per kilogram per 24 hours (creatinine coefficient). It is reduced in muscular dystrophy and with muscle wasting due to motor neuron degeneration. The reduction is in general proportional to the diminution of muscle mass

Normally, creatine output is about one-tenth that of creatinine in the adult male on a meat-free diet It increases whenever there is a marked degree of muscle breakdown or wasting, from any cause, or with certain metabolic disorders Creatinuria therefore serves as an index of the degree or nature of muscle involvement. Blood creatine may increase slightly but the blood creatinine remains normal Greater creatinuria is said to occur in children and some adult females without disease, but the output described above is usual for normal males.

Considerable creatinuria may occur during active wasting due to denervation (progressive muscular atrophy, poliomyelitis, nerve injury) or associated with arthritis or disuse atrophy. More interest, however, attaches to its occurrence with primary muscle disease, particularly muscular dystrophy.

Hoagland *et al* (86), who have taken up the study some 35 years after the original discovery of creatinuria in progressive muscular dystrophy, consider it to be one of the most striking manifestations of the disease. Even more important, in their opinion, is the diminished excretion of creatinine which provides a more reliable indication of the severity of the disease than does creatinuria, which may occur in many pathologic conditions not known to be associated

with muscle disease. In muscular dystrophy the residual muscle mass, as inferred from the excretion of creatinine, provides a useful index of the state of the disease at any given time. Milhorat and co-workers, in a series of papers (129, 132–34) have explored other aspects of the problem. In muscular dystrophy creatine tolerance is impaired and most of a 1 to 3 Gm. dose of creatine fed by mouth is excreted in the urine. A normal individual would store all this creatine in the muscles and excrete none in the urine. In general, it cannot yet be said whether the disturbance of creatine metabolism is merely an echo of muscle destruction or whether it plays a more primary and causative role. To some, creatinuria is to dystrophy what glycosuria is to diabetes. A marked increase in aminoaciduria in muscular dystrophy has recently been reported by Ames and Risley (4)

In myasthenia gravis and myotonia congenita, creatine and creatinine metabolism are normal Creatinuria may be present in myotonia atrophica, depending on the amount of atrophying muscle

Of special interest is the presence of creatinuria with a number of metabolic disorders in which muscular symptoms are prominent but actual wasting or destruction is absent Hyperthyroidism, Addison's disease, and eunochoidism in the male are characterized by creatinuria, and the same is true of animals which are fed thyroid extract, with adrenocortical deficiency, or after castration Suitable treatment abolishes the creatinuria. It is curious that each of the above conditions is associated with hypertrophy of the thymus We have studied the creatine output of rats injected with an extract of calves' thymus A marked increase of creatinuria occurred, but this was noted also in control animals injected with brain extract Finally, persistent creatinuria is an interesting finding in familial periodic paralysis, even between attacks It may indicate an underlying abnormality of muscle metabolism which, combined with a fall of blood potassium, results in the typical attack of paralysis

Electromyography Recording the electrical activity of muscle or of motor units or single muscle fibers is a valuable research method in the study of neuromuscular abnormalities In this way one can analyze not only the grosser phenomena of cramp, muscle spasm, or fasciculation, but the finer abnormalities of contraction which appear, for instance, in myasthenia gravis or myotonia In

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Fig. 8. Electromyography Photomicrograph of insulated needle electrode with bare tip inserted into fresh teased muscle fibers obtained by autopsy a, Needle electrode, b, A single muscle fiber

Fig. 9 Electromyograph being used on a patient with a median nerve injury.

like manner the effects of a test dose of a drug may be analyzed

Various types of recording can be employed. Surface electrodes may be attached on the skin over the muscle and they record gross changes A needle electrode may be inserted to tap a single motor unit or muscle fiber (Fig. 8). The tiny action currents are stepped up a million times or more and recorded visually on a cathode-ray oscillograph, or even heard through a loud speaker. Mechanical recording by ink-writer, etc., is too slow for some of the electric phenomena in muscle. A permanent record can be made, however, by photographing the oscillographic image at any given time.

Apparatus designed by Jasper and Forde (98) is in routine use at the Montreal Neurological Institute and is pictured in Figure 9. It should be stressed that this method has contributed little to the diagnosis of neuromuscular diseases This still rests on clinical grounds It is adding much, however, to our knowledge of mechanisms. Details of electromyography may be found elsewhere (44, 182)

In general, it can be said that a normal motor unit gives a definite pattern of electrical response to a willed movement. This consists of a series of triphasic waves, the negative spike being of greatest amplitude and reaching 2 to 6 millivolts Each wave complex is about 5 to 8 milliseconds in duration

A recently denervated muscle is silent About 2 weeks after degeneration of the motor nerve fiber, spontaneous fibrillation potentials appear These are also triphasic but of extremely brief duration (1–2 milliseconds) and small voltage (10–200 microvolts) They persist continuously and indefinitely in denervated muscle but disappear when reinnervation occurs They are thus of diagnostic and prognostic value

Complex and prolonged responses of polyphasic form (amplitude, 200–800 microvolts) are recorded from fasciculating muscle which is undergoing degeneration due to motor neuron disease Complete absence of potentials may occur, of course, in muscle which has become completely atrophied or dystrophic

Kugelberg (110) has shown that the earliest change in muscular dystrophy is the occurrence of large numbers of rapid action potentials with a duration of about 1 millisecond Later there is an inconstant diminution of amplitude and number of spikes This



Fig 10. Photomicrograph of section of muscle in myasthenia gravis. All muscle fibers are well preserved in appearance, equal in caliber, and show normal cross striations and normal capillaries lying parallel to each fiber; no evidence of degeneration or necrosis, no connective tissue or fatty infiltration.



Fig 11. Photomicrograph of section of muscle in thyrotoxic myopathy Three neurofibrilla (nf, slightly out of focus are lying over normal muscle fibrils One of these ends in a motor end-plate (mep), here seen as a cluster of dark gold chloride impregnated granules



Fig 12 Photomicrograph of section of muscle in progressive muscular atrophy One almost normal fiber, with cross striations still present, is seen lying between pale, degenerated "ghost fibers" in which cross striations are no longer evident The capillaries which lie parallel to the long axis of each normal fiber, are absent in many areas in this specimen A few small vessels lie over the fibers

seems to indicate that some of the muscle fibers served by motor units are gradually being wiped out, willy-nilly, by the disease process.

Muscle Biopsy We are increasingly impressed with the need for muscle biopsy in obscure cases of neuromuscular disease. Two cases of marked muscular fatigability in men of 38 and 45, respectively, due to generalized endarteritis of the smaller vessels, were only recognized in this way. The procedure has been of value even in better-known conditions. For experimental reasons we have made routine biopsies in most of our neuromuscular disease cases in the past few years

Punch devices are available but they do not provide enough tissue, and we take a sample through a small surgical incision, usually from the soleus muscle.

In addition to the usual formalin, alcohol, or Bouin's solution fixation, followed by paraffin-mounted staining with hematoxylin and eosin or hematoxylin and van Gieson's stain, fresh-teased preparations are also studied The latter method, described by both Carey (28) and Cole (35) as the "Gold Chloride Method for Motor End Plates," has proved superior in some respects to the paraffin method in that there is neither the shrinkage nor loss of substance and structure which occurs in the fixed specimens. The gold chloride sections may be teased out and studied within 24 hours of biopsy A greater number of representative samples of the specimens may be examined more rapidly than is possible by the paraffin method and the muscle can roughly be classified as "normal," "atrophic," or "dystrophic" In myasthenia gravis and in thyrotoxic myopathy, the teased sections appear normal (Figs 10, 11); in progressive muscular atrophy the morphologic changes vary greatly from one fiber to the next, so that many stages of the morbid process can be seen in one microscopic field This method also permits visualization of the capillary network In progressive muscular atrophy there is often a striking decrease in the number of capillaries (Fig 12) In the dystrophies, a capillary anastomotic network denser than normal is sometimes seen, in addition to marked infiltration of fat, whereas in the paraffin sections we have never been able to identify capillaries In dystrophy the profound changes in the fibers are not widespread and uniform, and are

Neuromuscular Disorders

NERVE CELL

Poliomyelitis. We consider here recent work on the pathophysiology of poliomyelitis in relation to the neuromuscular apparatus. No attempt is made to deal with great advances which have been made in the fields of epidemiology and virus research The polio virus has a selective affinity for certain nerve cells (17) and involves the brain as well as the cord in all cases Parts of the brain almost never affected are the cerebral gray matter (excepting the motor cortex), corpus striatum, and cerebellar cortex (excepting the roof nuclei), while the brain stem as far forward as the hypothalamus and thalamus bears the brunt of the attack. In the nerve cell the virus reacts primarily with the cytoplasm, and nuclear alterations occur secondarily.

Studies by Eben Carey and associates (29, 31) on motor end-plates carried out with the gold chloride method include visualization of the effects of poliomyelitis on the neuromuscular mechanism, both in the monkey and in man In Macacus rhesus there is first hyperemia and perivascular infiltration in the weakened but non-paralyzed muscles This is followed by retraction, hypertrophy, or granulation of end-plates at the onset of paralysis, and disappearance of about 50 per cent of end-plates within 4 days During the first week of paralysis masses of gold-staining axonic substance appear at the degenerating nerve ending and extend centripetally They seem to be due to unknown chemical reactions between virus and neuron at this level In 3 humans who died of bulbar poliomyelitis within 36, 96, and 168 hours after onset of symptoms, almost complete denervation of respiratory muscles was demonstrated within 26 hours As in the monkey, degeneration in the motor axons was centripetal

It is important to remember that in the early stages, probably up to 1 month, not all affected neurons are irreversibly damaged, some are merely sick and will recover Internuncial neurons in the cord and reflex pathways from the spinal ganglions may be injured, too, and doubtless add to the motor dysfunction

Occasionally in poliomyelitis there is widespread spasticity of skeletal muscles, as distinct from local muscle spasm This is thought to be a reflex phenomenon due to interference with inhibitory motor

sometimes spotty, as in the selective disintegration noted with progressive muscular atrophy By studying the fresh-teased gold chloride sections for morphology, and making parallel studies on the paraffin sections for cytology, an over-all picture is obtained

Biochemical Analysis of Muscle The results of biochemical analysis have been disappointing. To begin with, it is difficult to remove sufficient muscle from the living patient to permit determination of many substances, and autopsy material has doubtless undergone many changes Furthermore, even with the most favorable methods for rapid removal and fixation of material the more labile constituents such as glycogen, phosphocreatine, adenosine triphosphate, lactic acid, can change with bewildering rapidity. Finally, interpretation of the quantitative data is very relative since in a given sample, especially of diseased muscle, it is not known how much is fat, connective tissue, arterioles, venules, lymphatics, neurons, collagen, or perhaps even unknown end products of muscle metabolism in the living sarcoplasm.

Despite these criticisms, worthwhile determinations have been made in experimental muscular atrophy and nutritional dystrophy in animals These have been summarized in the respective sections A brief indication of the results in a few neuromuscular diseases is given in Table IV. Further work with a series of cases of these

TABLE IV

Biochemical Analyses of Biopsy Specimens in Neuromuscular Disorders (71, 139)*

Muscular dystrophy and dystrophia myotonica
- Total acid-soluble phosphorus ↘
- Creatine phosphoric acid content ↘
- Potassium content ↘

Myasthenia gravis
- Phosphorus holding compounds →
- Potassium content ↗

Progressive muscular atrophy
- Potassium content →

* The arrows indicate increase (↗), decrease (↘), or unchanged state (→) of the substance analysed

and other conditions, and with refined technics, seems indicated. The subject has been reviewed by Nevin (139) and by Goettsch *et al.* (71).

potentials found during regeneration of an injured nerve supply, during the active degenerative phase of progressive muscular atrophy, during spontaneous fasciculations of muscle and during both the acute and the recovery phases of polyneuritis Fasciculations were not affected by nerve block. They were exaggerated with neostigmine and were arrested by curare.

The presence of abnormal potentials indicating spasm in the intercostal muscles of patients in the acute stages of poliomyelitis is of prime importance and supports our belief that respiratory embarrassment is sometimes due to spasm and not to paralysis of respiratory muscles. In these instances vigorous hot packing of the chest may obviate use of a respirator. The use of drugs to reduce muscle spasm has been dealt with in the section on spasm.

One need in poliomyelitis is more specific knowledge of the relationship of spinal nuclei to the muscles which they innervate. Lesions of these centers could then be analyzed in terms of corresponding neuromuscular systems with definite reflex connections, so that the entire problem of port of entry and mode of transmission of virus, extent of neuronal damage, and indications for therapy might be clarified (56)

Progressive Motor Neuron Disease Under this heading are included progressive muscular atrophy, amyotrophic lateral sclerosis, and progressive bulbar palsy We look upon these three conditions as variants of the same disease. One or all forms may appear in the same patient during various stages of the disorder, and may provide a medley of signs pointing to both upper and lower motor neuron involvement Little has been added to our knowledge in recent years save that the highest levels of Betz's cells may be affected, that fasciculation is peripheral in mechanism, and that some mechanical lesions around the foramen magnum or cervical region occasionally simulate this syndrome

As regards the latter, most physicians are becoming acutely aware that some cases of spinal cord compression in the cervical region may give rise to a picture resembling amyotrophic lateral sclerosis Wasting and fasciculation in the hands plus pyramidal tract signs in the lower limbs may occur without manometric spinal block or signs of sensory involvement The temptation is to assume the presence of progressive motor neuron disease. Employment of contrast myelography solves this problem (24, 103)

mechanisms at the level of the brain stem Such spasticity has been produced experimentally by intracerebral inoculation of poliomyelitis virus in the monkey Histologic examination then shows lesions at the level of the reticular formation but no demonstrable lesions in the cord (18). Severe damage to the reticular formation in the brain stem of human polio victims who had marked spasticity before death has also been reported (9). Muscle spasticity has also occurred following reticulospinal tract section (176).

Electromyography is sometimes useful both in diagnosis and in determining the extent of denervation or spasm Fibrillation potentials may be recorded from a high percentage of paretic muscles from 3 weeks to a year after onset of poliomyelitis (93). This is, of course, an indication of denervation In old cases, stimulation of the motor nerve may result in an abnormal electromyogram which is corrected toward normal by neostigmine. Failure of some muscles to respond except after neostigmine may be due to abnormalities at the neuromyal junctions (87). Muscle spasm potentials may be present at rest, but more often appear when the muscle is stretched They not only occur in the antagonist of a weakened muscle but also in the paretic muscle The spasm is a reflex phenomenon and is not present in completely paralyzed muscle It can be stronger than the voluntary movements of which the same muscle is capable (159). Kabat and Knapp (100) claim that the spasm is largely abolished during spinal anesthesia Watkins *et al.* (180) found definite evidence of disordered reciprocal innervation in their study.

Jasper and Ballem (97), in this Institute, have studied the mechanism of muscle spasm in poliomyelitis by means of electromyography They conclude that all true spasm is of a reflex character, although fasciculations may be observed during the acute stage of the disease In some muscles which were in flaccid paralysis and which responded poorly even to galvanic current stimulation, no fibrillation could be detected and these muscles did not recover, even though they were not atrophied at the time of the first examination. This raises the question whether the disease may sometimes affect the muscle tissue itself, thus preventing denervation fibrillation.

Jasper (95) observed highly disintegrated polyphasic motor units during certain stages of poliomyelitis These were similar to the

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McCullagh and Hewlett (121) have described a case of acromegaly with proved (autopsy) amyotrophic lateral sclerosis, and two cases in which "amyotrophic" symptoms improved with treatment of the acromegaly. These cases are reminiscent of the changes in chronic thyrotoxic myopathy

The mechanism of fasciculation in progressive motor neuron disease is of interest Odom, Russel, and McEachern (141) have shown that fasciculation persists even after complete novocaine block of the motor nerve fibers going to the muscle As mentioned before, fasciculation should therefore not be considered as a sign that the anterior horn cells are dying off. Cramps and fasciculation are apt to come on during rest, often at night, and are dissipated or reduced after the patient has been up and about in the morning It is as if some chemical transmitter had accumulated during rest The fasciculation can be brought out or greatly intensified by a small dose (0 5 mg.) of neostigmine intramuscularly (141). Serum cholinesterase is normal in these cases but is of course reduced in the usual way by neostigmine (141).

Amyotonia Congenita This condition is due to lack of development or early degeneration of anterior horn cells in the spinal cord It is characterized by hypotonia, muscular weakness, and absence of tendon jerks from birth These features are not infrequent in hypotonic babies or those suffering from cerebellar birth injury or aplasia We have been fooled several times by finding later development normal in such children This is the only advance that we can report on Oppenheim's disease

NERVE FIBER

Lower Motor Neuronitis covers a number of unsorted conditions We prefer the term to "infectious polyneuritis," "Guillain-Barré syndrome," "Landry's paralysis," etc , because it denotes the unit most involved The condition certainly acts like a virus disease but differs from poliomyelitis in its symmetric involvement of the limbs, its often sporadic occurrence, and the tendency to complete recovery without residual paralysis The high cerebrospinal fluid protein without cellular increase is notable, but we should not forget that high protein levels in poliomyelitis often persist for weeks or months after abatement of the cellular reaction Meningeal signs and spasm of limb muscles may be just as prominent as with the other disease,

and they respond to the same treatment. We have studied one case, in a boy, which was so gradual in onset and so chronic that a diagnosis of progressive muscular dystrophy was made Later a spinal puncture was done and the fluid found to have a very high protein level. Recovery was ultimately complete Cases associated with acute porphyrinuria have been ably discussed by Denny-Brown and Sciarra (47). A simple method for the examination of urine for porphyrins has been described recently (41).

NEUROMYAL JUNCTION

Myasthenia Gravis. This extraordinary disease is characterized by weakness and undue fatigability of the skeletal muscles Heart and visceral musculature are not involved The muscles innervated from the brain stem are first, and most, affected. The patient is thus apt to have drooping eyelids, a blank, expressionless appearance, a nasal voice, and difficulty in chewing and swallowing In severe cases there may be superadded weakness and fatigability of the limb muscles so that arms and legs can scarcely be lifted. In extreme cases the respiratory muscles may be affected, and the patient dies of respiratory paralysis or spasm of the larynx The above features resemble unmistakably the acute and evanescent effect of curare poisoning.

The general character of myasthenia gravis is suggestive of an endocrine or metabolic disorder Onset is usually after puberty and in early life There is a tendency to remissions Modifications of the disease may occur during pregnancy or with changes of thyroid function At autopsy no structural change is found in the nerves or muscles except for the presence of scattered lymphorrhages, which can scarcely be considered causative. The only other common finding is hyperplasia or tumor formation in the thymus, which occurs in a suggestive number of cases—in about 50 per cent of reported autopsies (weighted) and about 10 per cent of surgical explorations in unselected cases Even the latter figure is high

There are other similarities too between myasthenia gravis and curare poisoning Numerous myographic and electromyographic studies (83, 84, 141) indicate that in both conditions there is a sort of block to normal neuromuscular function The motor nerve fibers conduct normally, and the response of skeletal muscle to direct

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Thymectomy was first embarked upon in an organized way by Blalock of Johns Hopkins Hospital (16), and has now been carried out in several hundred cases Keynes (106), the surgeon, has himself completed 100 thymectomies. He has summed up his results (1942–1947) as follows:

Patients	No.
Assessed	63
Untraced	1
Recent	18
Died postoperatively	8
With thymic tumors	10
Total	*100*

Only one of the patients with thymic tumors showed improvement, and five had died by the end of the third postoperative month These results are odd. In the 63 nontumor patients whom Keynes assessed the results were as follows:

Assessment	No	Per cent
A. Well. No neostigmine	24 }	63
B. Almost well	16 }	
C. Better	18	29
D. No change	5	8
Total	*63*	

The Mayo Clinic experience has been summarized by Eaton (51) He analysed results in 52 operated cases, of whom 20 had thymic tumors and 32 had no tumors Results were compared with 128 control cases seen in the same period of time All cases were studied for one year or longer before the evaluation was made There were 8 per cent remissions in each group However, 50 per cent of the operated group had shown unequivocal improvement while only 25 per cent of the control group had shown similar improvement

Harvey (80) has recently reviewed the experience with 125 patients with myasthenia gravis at Johns Hopkins, of whom 32 underwent thymectomy His studies indicate that beneficial results are greater than might be expected from spontaneous remission He

stimulation is normal, but the transmitting machinery from motor end-plates to muscle does not function properly. Furthermore, both conditions respond dramatically to anticholinesterase drugs such as eserine and neostigmine. The myasthenic patient is made much worse by small doses of curare.

Let us examine the various possible derangements that might occur in the chemical transmitting mechanism from nerve to skeletal muscle.

(*1*) *Deficient synthesis or liberation of acetylcholine at motor end-plates* This is a popular theory and may well be the correct one, but it is difficult to prove or disprove The beneficial effects claimed for acetylcholine and other more stable choline esters (64) has not been confirmed by others (108) or by our own experience. Further critical experiments with newer technics may give the answer one way or another.

(*2*) *Increased breakdown of acetylcholine due to overactivity of the enzyme, cholinesterase.* It has been shown by a number of workers that blood cholinesterase is normal in myasthenia gravis (141). Muscle cholinesterase has also been found normal in the few cases where the determination has been made (122).

(*3*) *Diminished sensitivity of muscle to acetylcholine* Here we run into the confusing observation that myasthenic muscle, unlike curarized muscle, is actually hypersensitive to acetylcholine injected into the nutrient artery (111). This could mean, of course, that the two conditions are quite different in mechanism On the other hand, curare poisoning is an acute affair whereas myasthenia gravis is chronic. We know that denervation of a muscle results, after a couple of weeks, in increased sensitivity of the muscle to acetylcholine Could it be possible that in myasthenia gravis some long acting, curare like substance produces a chemical denervation, with resulting sensitization of muscle to acetylcholine? No answer is at hand on this score

(*4*) *Presence of curare like barrier at the neuromyal junction* There is no proof of this It has been reported (178) that release of the tourniquet from an exercised limb in a myasthenic patient causes weakness of relatively unaffected muscles elsewhere in the body The suggestion is that some curare like substance has been released from the exercised muscle into the general circulation These experiments are inconclusive, and in any event might point only to the release of normal metabolites with momentary effect on muscles elsewhere It is tempting, of course, to think of the thymus as perhaps elaborating some curare like substance with resulting effect on the musculature throughout the body. Experiments done by us have been cited elsewhere (122) There is as yet no experimental evidence of such a thymic function, and the nearest clinical evidence is provided by the somewhat encouraging results of thymectomy.

that tests be perfected for neostigmine in blood and urine, so that the fate of the drug can be accurately traced. We may also have been misled into thinking that the drug acts only through its anticholinesterase power. Some drugs, like DFP. (36,81), are less effective, even though they are more powerful anticholinesterases. Neostigmine, by the way, can be given intravenously without atropine and over the course of 1 minute, as a rapid and decisive test for myasthenia gravis The dose is 0 5 mg , and symptoms begin to clear in about 2 minutes (173).

Congenital and/or neo-natal myasthenia gravis is of great interest and may point to important mechanisms. We have studied a female patient, in her twenties, with severe myasthenia gravis for several years Thymectomy was performed 2 years ago without improvement. A year ago she became pregnant and ran perilously through the course, without the usual remission The baby was born at term with a fantastically short and easy labor Dr. A. K Geddes, the pediatrician in charge, noted at once that the child was hypotonic and listless, the eyes were not held closed, breathing was weak, and accumulation of heavy mucus in the respiratory tract was a constant menace. An intramuscular injection of 0 1 mg neostigmine caused prompt improvement. Maintenance therapy with neostigmine, and vigilant nursing were necessary until the thirty-fifth day, when the child was able to subsist normally without neostigmine In the following 2 weeks the baby became "hypertonic" with some generalized muscular rigidity and increased tendon reflexes This was thought to be a neuromuscular disorder and not evidence of cerebral damage This view seems to have been correct, since the baby has now become normal. The hypertonia was like a back-swing from the myasthenic state The mother died on the thirty-fifth post-partum day, apparently due to an increase of her myasthenic symptoms

This case, of course, brings up the point as to whether the mother may have been passing through the placenta to the foetus some long-lasting, curariform substance. Other theoretical interpretations are possible Levin (114a) has recently reviewed this subject. Dr Geddes will report his own carefully documented case

Some patients with myasthenia gravis present a picture of restlessness, emotional lability, sleeplessness, and anxiety that is quite extraordinary. It is popular to ascribe this to emotional difficulties

suggests that our modern neostigmine therapy may interfere with spontaneous remissions of the disease.

It can only be said that in an encouraging number of cases there has been improvement, amounting in some instances to cure The disturbing fact remains, however, that some patients are benefited little, and others not at all This is hard to reconcile with the view that the thymus is primarily responsible, unless it is argued that the surgeon has overlooked some aberrant or hidden thymus tissue This fault he makes every effort to avoid. Castleman (32) has recently reported that there is a characteristic histologic picture in the thymus which can be correlated with the presence of myasthenia gravis.

The study covers 35 cases of myasthenia gravis in which the thymus was investigated in detail Tumor was found in 10 instances In the majority of the remaining cases the thymus was grossly normal as regards size and weight, but microscopic examination showed varying degrees of lymphoid germinal center infiltration of the medulla Germinal centers are not usually found in the normal thymus

The morbid anatomy of the endocrine glands should be reviewed by some bright pathologist with the total picture of the disease in mind, and with the new technics, or even newer ones that can be devised That enigmatic organ, the thymus, should be attacked with fresh staining technics, and other glands, too, since the condition may be a polyglandular one Above all, that citadel gland, the pituitary, should be examined carefully in these cases, with accurate differential cell counts Cushing established the entity of pituitary basophilism out of a confused welter Adenomas of the adrenal cortex are common in that disease, although not causative Thymic tumors might bear a similar relationship in myasthenia gravis

We know little about the fundamental effects of neostigmine in myasthenia gravis A tablet or two, taken by a normal person, will cause distressing neuromuscular and visceral effects The myasthenic patient may take 20 or more tablets in a day and feel only benefit to his muscle power and no visceral effects It is almost as though his system had a deficiency of the drug and lapped it up With improvement of the myasthenia in a spontaneous remission, his body may soon tell him that the dose should be cut It would be desirable

to keep it in sealed ampoules or small, rubber-stoppered bottles for intramuscular or oral use, respectively. 10 mg. of TEPP. by mouth is equivalent to 100–150 mg neostigmine given by mouth. The drug should not be used until the effects of previous neostigmine therapy have worn off (24 hours) and the two should not be used together

The Myotonias Myotonia is a symptom It consists of the prolonged contraction of a muscle or part of it after cessation of the stimulus—be it voluntary, mechanical, or electric With a voluntary movement a whole group of muscles may remain in contraction for some seconds before relaxation sets in A similar but very localized contraction follows local stimulation of the muscle by percussion, an electric shock, or the prick of a needle. When a movement is repeated a number of times the muscles "warm up" and normal contraction and relaxation may occur The myotonia returns again after a period of rest Myotonia may be demonstrable clinically in all the voluntary muscles, or it may be limited to a few, such as the thenar muscles and tongue In such cases, however, the more refined method of electromyography sometimes shows that the myotonia is more widespread Denny-Brown and Nevin (45) suggest that the generalized hypertrophy of muscles which occurs in some cases of myotonia may be due to reflex "after spasm" in muscles not otherwise myotonic

Myotonia occurs with two rare but well-recognized diseases—myotonia dystrophica and myotonia congenita It has been reported also in association with myxedema or hypothyroidism, and in these instances it responds to treatment with thyroid It also occurs in goats as a hereditary disease which seems identical to myotonia congenita in humans The phenomenon of myotonia is of similar character in all these conditions

Myotonia congenita (Thomsen's disease) is a heredofamilial, nonprogressive condition characterized by mytonia and sometimes by generalized, true muscular hypertrophy It may be recognized at birth, or it may make its appearance at puberty Under the microscope the muscle is seen to be paler than normal and shows hypertrophy and sometimes an increase of sarcolemmic nuclei

Myotonia dystrophica (Steinert's disease) may occur sporadically or with a heredofamilial background It usually appears, or is first recognized, in adult life and is characterized by myotonia and by

or to the acute distress of muscular weakness. Often, however, it appears to be part of the disease, and it certainly can flourish at a time when muscular symptoms are under good control It is very reminiscent of the state often seen with Graves's disease. It seems naive to attribute it to some organic hypothalamic upset when there is probably a maiden aunt in the background of some forgotten pubescent day, yet these episodes are part of the picture of the disease and should be assessed from that viewpoint too. Curare can also produce central effects, first of somnolence and, with larger doses, anxiety and tenseness.

In the therapeutic field things are much the same. Roentgenotherapy to the thymus appears to be unprofitable. Thymectomy is a hopeful thing, to be conjured with. Neostigmine is the mainstay Ephedrine is an indispensible adjuvant which prolongs and fortifies the neostigmine effect, and the two should be used together. Guanidine, potassium, and DFP have some influence, but have been largely abandoned for clinical use.

Adrenocorticotrophic hormone (ACTH) has been tried by Torda and Wolff (173a) in 5 cases During a 5-day period of therapy with 80 mg per day, there was a slight increase of muscular disability along with other generalized symptoms 2 or 3 days after cessation of treatment, there began an improvement in the muscular fatigability, which the authors interpreted as a partial remission of the disease. Reference will be found in their paper to a great body of previous work done by them, which suggests that there may be a deficiency of acetylcholine synthesis in myasthenia gravis

Tetraethylpyrophosphate (TEPP) is a new and promising agent for the treatment of myasthenia gravis This potent anticholinesterase has neuromuscular and visceral actions similar to neostigmine and DFP Its action time, however, is intermediate, with the rough ratio of neostigmine 2–3 hours, TEPP 24–48 hours, DFP 7–14 days Burgen *et al* (25b) studied the effect of TEPP in 3 myasthenic patients and found that it gave more even control than neostigmine. Patients with predominant limb weakness respond better than those with ocular and bulbar symptoms The visceral effects are controlled by atropine in the same way as with neostigmine

TEPP is rapidly broken down by contact with water It is therefore customary to dissolve it in anhydrous propylene glycol and

costerone are among the agents capable of producing a fall of serum potassium and paralysis in persons subject to periodic paralysis

Lilienthal and others (57a) have shown that wide variety of drugs and biochemical agents can cause myotonia and repetitive phenomena Among these is adenosinetriphosphate (ATP.). This natural inhabitant of muscle, along with a proper concentration of the potassium ion, is necessary for the contraction of the actomyosin complex, according to Szent-Györgyi (171). When one considers the sensitivity of myotonic muscle to potassium and the disapperance of myotonia with low potassium levels, the opposite role of ATP. concentration becomes alluring. Unfortunately, little is known of this in disease. The compound 2,4-dichlorophenoxyacetate (2,4-D) causes typical myotonia in the rat, and this can be abolished by alpha-tocopheryl phosphate

Kennedy and Wolf (104) suggested that myotonia and myasthenia gravis were clinical opposites because of their opposite reactions to neostigmine and to quinine They even went so far as to cross-transfuse two patients who had the respective diseases No improvement occurred in either. The concept is interesting but does not fit all the facts. The supposition that myotonia may be due to increased liberation of acetylcholine at the neuromyal junctions has no proof to support it.

The improvement of myotonia by quinine is due to its ability to suppress repetitive muscle responses of all types, and also by its curarizing action, which diminishes the number of impulses reaching the muscle fiber (77). Quinine is prescribed clinically for its symptomatic effect in reducing myotonia Calcium diminishes or abolishes myotonia in goats (66)

MUSCLE

Muscular Infantilism Gibson (70) has described a patient with deficient muscle power first noted in childhood. The condition was hereditary and occurred in 14 of 26 male relatives liable to be affected and in 7 of 21 female relatives It was nonprogressive and was characterized by creatinuria, but muscle biopsy showed relatively normal fibers At the time of Gibson's examination, the patient was 26; when seen 2 years later the condition was the same. We have recently examined the same patient at the age of 57 His muscle condition shows no change.

progressive dystrophic wasting of certain muscles without fasciculation. This occurs predominantly in the facial muscles (myopathic facies), Sternomastoids, and peroneal muscles. Associated changes are cataracts, frontal baldness, testicular atrophy, and impotence. Microscopic examination of the muscle shows degenerative changes characteristic of dystrophy. Maas and Paterson (117) have claimed that both the above diseases are simply early and late manifestations of the same condition, but most workers consider them to be separate entities.

What is the nature of myotonia? This has been a matter of debate for many years Some investigators have considered it to be a form of contracture (without repetitive action currents) similar to that produced by a large, intra-arterial injection of acetylcholine in the experimental animal, others have looked upon it as a tetanus of peripheral origin, or due to reflex or central stimulation. Myotonia persists after blocking of the motor nerve (157) and after spinal anesthesia (104), thus indicating a peripheral mechanism Further investigation, using electromyographic methods, has shown that the abnormality lies predominantly or altogether in the muscle. The difficulty in willed relaxation is not due directly to the peripheral myotonia but to reflex (central) spasm of the prime movers and synergists (45)

Brown and Harvey (23) have been able to study the mechanism in myotonic goats in which the clinical features and response to drugs are exactly the same as in the myotonic human The condition, as studied in electromyograms, is a long-lasting, irregular tetanus. The response of the muscle to a single motor nerve stimulus is repetitive in nature The myotonic response to mechanical stimulation is not diminished by full curarization, nor by section and degeneration of the motor nerve Response and threshold to acetylcholine is the same in myotonic and normal goat muscle, but the response is much prolonged in the myotonic.

There are interesting relationships to potassium Myotonic muscle is abnormally sensitive to the potassium ion Epinephrine, which lessens the stimulation of striate muscle by potassium (79), reduces the myotonia of goats and of humans (150) Desoxycorticosterone, which influences potassium balance and lowers serum potassium, abolishes myotonia in the goat Both epinephrine and desoxycorti-

next to several normal ones. The combination of normal fibers with abnormal fibers in various stages of degeneration, down to actual fibrous or fatty replacement, is characteristic. The earliest change is a lack of clarity or disappearance of cross striations with swelling, vacuolization, and altered staining reactions in the sarcoplasm. Later, the sarcolemmic nuclei proliferate, phagocytes enter, and completely new tissues—fat and fibrous tissue—appear (139,187).

In sections made from muscle biopsies from our dystrophic patients, marked changes in vascular patterns have been found. This is particularly noticeable in the fresh-teased preparations stained by gold chloride The capillary anastomotic network is denser, coarser, and more profuse than normal Arterioles show thickening and proliferation of the medial coat and adventitial coverings Sometimes complete obliteration of the lumen is observed It is not possible to say whether these vascular changes are primary or secondary to changes in the muscle fibers

It is of interest that Holman and Swanton (89) produced degenerative lesions in the arteries of young dogs by a combination of renal injury and a deficient diet It was found that addition of mixed natural tocopherols to the diet prevented the appearance of the experimental necrotizing arteritis (88)

From the biochemical viewpoint, creatinuria is the most characteristic finding This has been discussed in the section on creatinuria. Chemical analyses of dystrophic muscle have been reviewed by Nevin (139). A lowering of various compounds has been reported, e g, creatine, acid-soluble phosphorus, phosphocreatine, adenosine triphosphate These changes indicate merely the loss of specific muscle compounds and are hard to relate to the disease Nevin's demonstration, in muscle biopsies from 4 cases of dystrophy, that the breakdown and resynthesis of phosphate compounds occurred almost as readily as in normal muscle, is of interest The conclusion is that no essential biochemical change has yet been uncovered in this disease

The most recent large-scale study of the condition has been made by Shank, Gilder and Hoagland (162) *Their results, which are* summarized below, give a picture of our present knowledge of the problem from the clinical and metabolic viewpoint

Their study of 40 patients with progressive muscular dystrophy gave evidence of the hereditary nature of the disease, 14 of the

Whether the above is a nonprogressive form of muscular dystrophy is hard to say. The musculature is pretty generally involved. We have seen several cases of progressive muscular dystrophy in adults where the progression of symptoms has been slow, covering a period of 20 to 30 years, and still does not cause complete disablement

Progressive Muscular Dystrophy. In this condition the muscle fibers undergo slow degeneration with ultimate replacement by fat and fibrous tissue The trunk, girdle, and proximal limb muscles are mainly involved, while the distal muscles of hands and feet are relatively spared Males are affected about five times as often as females There is a strong tendency to familial involvement but many sporadic cases are seen.

Julia Bell's careful scrutiny of 1,228 cases from the literature and 113 further cases from the records of the National Hospital, Queen's Square, London, has shown that the condition is simple recessive or sex-linked recessive when hereditary (10).

In the past, innumerable groupings have been made (e g , facio-scapulo-humeral, pseudohypertrophic, etc) according to differences in the location of the wasting and age of onset. It does not seem profitable to perpetuate these nosologic refinements unless it can be shown that the groups differ from one another in the cause or mechanism of dystrophy With our present knowledge the essential process appears to be similar in all (6)

Clinically, there is proximal weakness in the lower extremities, a waddling, unsteady gait with a broad base stance, bulging muscles, particularly the gastrocnemei, and, in the erect position, a marked lumbar lordosis There is progressive involvement of the arm and shoulder girdle muscles affecting the infraspinati and supraspinati, pectoral, latissimus dorsi, rhomboid, biceps, and triceps The tendon reflexes become hypoactive and eventually disappear, with the ankle jerks going last There is no fasciculation Response to galvanic and faradic currents diminishes late in the course of the disease There are no sensory disturbances and electric signs of nerve degeneration are absent Prognosis is grave because of low resistance to intercurrent illnesses and ultimate helpless invalidism

From the pathologic viewpoint the process is confined to the muscles, and changes are not found in the other organs In the early stages patchy degeneration appears, one affected fiber lying

next to several normal ones The combination of normal fibers with abnormal fibers in various stages of degeneration, down to actual fibrous or fatty replacement, is characteristic. The earliest change is a lack of clarity or disappearance of cross striations with swelling, vacuolization, and altered staining reactions in the sarcoplasm Later, the sarcolemmic nuclei proliferate, phagocytes enter, and completely new tissues—fat and fibrous tissue—appear (139,187).

In sections made from muscle biopsies from our dystrophic patients, marked changes in vascular patterns have been found. This is particularly noticeable in the fresh-teased preparations stained by gold chloride The capillary anastomotic network is denser, coarser, and more profuse than normal. Arterioles show thickening and proliferation of the medial coat and adventitial coverings Sometimes complete obliteration of the lumen is observed It is not possible to say whether these vascular changes are primary or secondary to changes in the muscle fibers

It is of interest that Holman and Swanton (89) produced degenerative lesions in the arteries of young dogs by a combination of renal injury and a deficient diet It was found that addition of mixed natural tocopherols to the diet prevented the appearance of the experimental necrotizing arteritis (88).

From the biochemical viewpoint, creatinuria is the most characteristic finding This has been discussed in the section on creatinuria. Chemical analyses of dystrophic muscle have been reviewed by Nevin (139) A lowering of various compounds has been reported, e g, creatine, acid-soluble phosphorus, phosphocreatine, adenosine triphosphate These changes indicate merely the loss of specific muscle compounds and are hard to relate to the disease Nevin's demonstration, in muscle biopsies from 4 cases of dystrophy, that the breakdown and resynthesis of phosphate compounds occurred almost as readily as in normal muscle, is of interest. The conclusion is that no essential biochemical change has yet been uncovered in this disease

The most recent large-scale study of the condition has been made by Shank, Gilder and Hoagland (162) Their results, which are summarized below, give a picture of our present knowledge of the problem from the clinical and metabolic viewpoint.

Their study of 40 patients with progressive muscular dystrophy gave evidence of the hereditary nature of the disease, 14 of the

patients were from families in which other cases of the disease had occurred. The early onset of the disease was demonstrated, the first symptoms having been noted before the age of 10 years by 62 per cent of this series of patients. The course of progressive muscular dystrophy was most rapid in patients with onset of the disease before the fifth year of life.

There were characteristic changes in the roentgenograms of patients with progressive muscular dystrophy. These alterations included conspicuous streaking of the soft tissue shadows of affected muscle, delayed appearance of centers of ossification in the bones of the hands and in the epiphyses of the long bones, and dimineralization of other bony structures.

The rate of excretion of creatine was greater, and the rate of excretion of creatinine was less, in boys with muscular dystrophy than in normal boys of the same age group who were maintained on diets of identical composition. These differences were increased when the subjects were fasting. The concentration of creatine in the plasma of dystrophic children was greater than in the plasma of normal subjects Levels of creatinine in the plasma were unchanged. The creatine tolerance of children with progressive muscular dystrophy was not significantly different from that of normal children of the same age group

The basal metabolic rate was low in children with the disease The median basal metabolic rate for a group of 14 patients was −14 5 per cent of normal standards The fasting level of the blood sugar, and the response to intravenous administration of dextrose, were essentially the same in dystrophic patients as in normal subjects Patients with progressive muscular dystrophy were in positive nitrogen and phosphorus balance

Menopausal Muscular Dystrophy This is a term that we have coined to describe 7 female patients who developed muscle weakness of severe degree at about the climacteric age Muscle weakness involved all extremities, but was most marked proximally and especially in the hip-girdle and thigh muscles Several had been victims of hyperthyroidism, and the condition persisted after thyroidectomy Of 5 treated with wheat germ oil, 3 showed remarkable recovery and returned to normal activity There is little doubt that some myopathies are remediable Perhaps all will be in time

Dermatomyositis. This condition which affects muscle, skin, and collagen tissue is a rare disease of multiform character. It may occur in acute or chronic form. It may be fatal, or recovery may be complete. Some recovered cases show permanent muscular atrophy and skin changes Common early symptoms are muscular pain and weakness, or edema and skin changes. Sometimes both occur together. Proximal muscles of the limbs are apt to be most involved. The muscles may be flabby, or tough and rubbery like an automobile tire. Muscles innervated by the brain stem may also be involved, with consequent dysphagia, dysphonia, etc. Skin changes are often similar to those of scleroderma. Erythema and pigmentary changes reminiscent of pellagra may occur on exposed areas. Creatinuria is common, as might be expected with destruction of muscle

O'Leary and Waisman (143) have made a study of 40 cases. Diffuse alopecia, stomatitis, and subcutaneous calcification occurred in some of their patients Moderate eosinophilia was found in 11 patients Histologic studies were made on skin and muscle from over 20 patients. The muscle specimens showed parenchymal and interstitial changes of nonspecific character. No special mention is made of the reactions of blood vessels supplying skin and muscle.

Despite the title, dermatomyositis may not be an inflammatory disease. It could indeed be due to nutritional deficiency Milhorat (131) has reported 2 cases improved by wheat germ therapy, and all of our 3 cases improved markedly with wheat germ oil (see section on Nutritional Muscular Dystrophy).

Lepine (114), in 1901, first conceived the idea that dermatomyositis may be primarily a disturbance of the blood vessels Silverman and Powell (164) found many changes in muscle fibers and also small round cells about the medium-sized vessels, but no proliferative endothelial alteration

MISCELLANEOUS

Thyroidal Neuromuscular Disorders (*1*) *Hyperthyroidism* Muscular weakness and fatigability may be prominent in uncomplicated hyperthyroidism The mechanism is not clear but it seems likely that there is direct embarrassment of muscle metabolism. Certainly creatinuria is marked, and muscle isolated from a hyperthyroid animal uses much more oxygen than normal (123). In addi-

tion, however, there are a number of well-defined neuromuscular disorders which may coexist with hyperthyroidism and which may be modified by it A study of the interrelationships between these conditions might well shed light upon them or, even more important, upon the manifold problems of muscular fatigue in man

The following neuromuscular disorders which may appear with the hyperthyroid state are described briefly in order to emphasize the wide variety of mechanisms that may be involved.

(a) *Exophthalmic Ophthalmoplegia* (20,127). This consists of progressive protrusion of the eyeballs due to a dystrophic process affecting the extraocular muscles. There is edema, fatty infiltration, and swelling of the muscle fibers. The resultant impairment of eyeball movements does not respond to neostigmine. The condition is apt to appear or become aggravated when the hyperthyroidism is brought under control For this reason, and because of numerous experimental facts, the anterior pituitary thyrotropic hormone is suspect rather than the thyroid itself No satisfactory medical treatment has been developed, and surgical decompression of the orbit may be necessary to preserve vision.

(b) *Acute Thyrotoxic Encephalomyopathy* This jaw-breaker title covers fairly completely a condition recently described in the Scandinavian literature (177) It results in a picture of bulbar palsy sometimes associated with Parkinson-like tremors and delirium or coma It is relieved by treatment of the hyperthyroidism

(c) *Chronic Thyrotoxic Myopathy* (125). It is characterized by generalized weakness, wasting, and fasciculation of the voluntary muscles This picture, which resembles so closely the incurable progressive muscular atrophy, vanishes after subtotal thyroidectomy. The condition is apt to occur in older people and without the usual florid evidences of hyperthyroidism such as goiter, exophthalmos, or tachycardia The large and violent twitches of muscles in the lower limbs may "throw" the patient and cause him to fall

The question sometimes arises as to whether fasciculation of muscle may itself give a falsely high basal metabolic rate We have studied 9 cases of progressive motor neuron disease with brisk fasciculations, and the basal metabolic rate was normal in 7 instances (125) Fasciculation cannot therefore be held responsible for the increased metabolism in cases of chronic thyrotoxic myopathy Mus-

cular rigidity and tremor as seen in Parkinsonism may cause a large increase in basal metabolic rate. This usually falls to normal when the test is repeated 2 hours after an oral dose of sodium amytal (6 grains) or immediately after an intravenous injection of myanesin

(*d*) *Periodic Paralysis* (147) This condition is really a disorder of potassium metabolism in relation to neuromuscular function. Recurrent attacks of flaccid paralysis occur, lasting many hours and disappearing spontaneously. Attacks are associated with a fall of serum potassium, and they are rapidly terminated by administration of potassium salts These bouts of paralysis are greatly increased in frequency and severity in the presence of hyperthyroidism or with thyroid feeding In Shinosaki's (163) series of 24 cases, 7 had hyperthyroidism and 8 simple goiter In Tsuji's (174) group of 14 cases, 11 showed evidence of hyperthyroidism Whether the so-called Basedow's paraplegia described in the European literature represents the same thing is not clear. See also page 254

(*e*) *Myasthenia Gravis* (122) The association of hyperthyroidism with myasthenia gravis has rarely been reported. We have assembled data from 8 cases (including 2 of our own) for which adequate information has been reported (124)

It is noteworthy that all of these patients have been women and that their ages ranged from 20 to 59 years Initial basal metabolic rates ranged from + 21 per cent to + 52 per cent before any treatment was instituted for the hyperthyroidism Symptoms and signs of muscle weakness were characteristic of myasthenia gravis and responded in all instances to neostigmine Of the 4 instances in which myasthenia gravis appeared before the hyperthyroidism, the later appearance of hyperthyroidism brought unmistakable improvement of the symptoms of myasthenia in 2

For the most part there seems to be an inverse, "see-saw" relationship between the two conditions—the myasthenia waning with the onset of hyperthyroidism or waxing with the treatment of the hyperthyroidism. This relationship was observed in both of our cases and was illustrated on 5 occasions in our second case.

(2) *Hypothyroidism* Myotonia has been repeatedly described both in cretin infants and in adults following thyroidectomy The symptom is relieved by thyroid administration. This subject has

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Attacks can be produced by the administration of epinephrine, glucose, or adrenocortical extract. All of these methods seem to involve the carbohydrate cycle. There is no diuresis of potassium prior to or during attacks. It seems that potassium is called from the blood to meet a need in the muscles.

Not all patients with a low blood potassium develop periodic paralysis. It is well known that some diabetics under insulin-glucose therapy show a sharp drop in serum potassium. Muscular weakness develops in some, however, and potassium is now included in the armamentarium for treating diabetic coma. Overtreatment of Addison's disease with desoxycorticosterone acetate may also cause a fall in serum potassium. We have seen several cases with no paralysis despite a serum potassium level below 10 mg. per hundred cubic centimeters. Ferrebee *et al* (60) described the equivalent of periodic paralysis in dogs subjected to large doses of desoxycorticosterone acetate. In essence, there seems to be some fault of muscle metabolism too in those who develop paralysis with the fall in blood potassium. Several patients subject to periodic paralysis have shown persistent creatinuria in between attacks.

Hubert Jantz (94), in a study of 9 cases of periodic paralysis, found that between attacks all of the serum potassium was ultrafiltrable but during the paralysis only a portion, sometimes less than half, was found in the ultrafiltrate The potassium concentration of muscle, taken by biopsy during the height of a severe attack, was 710 mg per 100 Gm Another muscle sample taken from the same patient 30 minutes after recovery contained 360 mg per 100 Gm. It seems that skeletal muscle, under some circumstances, has a need for more potassium and that it then drains the blood of its supply. This mechanism must surely operate to some extent in health, and perhaps in a wide variety of metabolic states that lead to minor fatigue and weakness Jantz thinks that lack of ionized potassium inhibits the normal cleavage of phosphocreatine in muscle. If correct, this would do more to explain the mystery of periodic paralysis than anything yet proposed.

(2) *Sporadic Periodic Paralysis.* We think that this occurs quite often but is hard to prove One patient with no familial history had definite attacks of periodic paralysis. Some mornings weakness of the limb muscles was so great that he could not put on his coat or

been reviewed by Poncher and Woodward (146) and by Nick (139a)

Periodic Paralysis. (*1*) *Familial Periodic Paralysis* This rare disease is characterized by recurrent attacks of flaccid paralysis affecting mainly the muscles of the trunk and limbs, during which the deep reflexes disappear and the muscles become inexcitable to electric stimulation. Attacks usually come on during sleep, without previous warning; they last from a few hours to 3 or 4 days In the intervals, muscular power is normal and the patient is entirely well Attacks first make their appearance during childhood or adolescence and become less frequent, or disappear, in later life. Males are twice as often affected as females Sporadic cases have been reported, but the condition is usually hereditary; Holtzapple (90) has described 17 cases in 4 generations of the same sibship. Many theories have been advanced to explain these weird and crippling seizures

The most comprehensive study of the disease has been made by Shinosaki (163) who published his observations on 24 cases Among the more important observations were the frequent (62 per cent) association of the disease with thyroid struma and the tendency for attacks to occur after administration of thyroid, epinephrine, or diets high in carbohydrate. Hyperglycemia was often observed during the early stages of spontaneous attacks, and albuminuria was present during attacks in 73 per cent of the cases Shinosaki carried out many ingenious experiments and concluded that the disease was a polyglandular syndrome in which the thyroid played an important part

After some preliminary leads, a number of workers (1,3,60,65, 147) pinned the condition down to an aberration of potassium metabolism, and the whole history of this development will be found in the reviews of Talbott (172) and Gass *et al.* (67).

Attacks of paralysis are associated with a drop of the serum potassium level from the normal of 18 to 22 mg. per hundred cubic centimeters to as low as 5 mg per hundred cubic centimeters, but there is no critical level for onset of muscular weakness. Recovery occurs rapidly when a potassium salt is given in adequate amount (intravenously, in 5 to 60 minutes, orally, in 1 to 5 hours). There is nothing more dramatic in medicine, and the usual hoodoo of "hysterical paralysis" is quickly dissolved when the real mechanism is known.

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(2) *Sporadic Periodic Paralysis.* We think that this occurs quite often but is hard to prove. One patient with no familial history had definite attacks of periodic paralysis Some mornings weakness of the limb muscles was so great that he could not put on his coat or

shave unassisted. Serum analysis at the time of weakness showed a low potassium level (13 mg./100 cc.), both the potassium and the muscle power returned to normal after administration of potassium chloride by mouth. An interesting finding grew out of this The patient had learned by experience that going out "with the boys for a beer" the night before prevented any muscular weakness from appearing the next day. A request for information from the brewing company showed that 1 quart of the favorite ale contained the equivalent of 1 Gm. of potassium chloride.

We have examined 2 girls in their early teens, each of whom suffered rapid onset of generalized muscular paralysis within a few hours Both had rcovered at the time of examination, 24 to 48 hours later. In each instance, no residual sign of central nervous system defect could be found. The cerebrospinal fluid was normal and there was no suggestion of poliomyelitis. Hysteria was invoked without cause. We suspect these were isolated episodes of periodic paralysis Both young women developed symptoms as a result of exposure to cold, and both at the time of a menstrual period. Serum potassium was normal in both instances at the time of examination Shinosaki showed that exposure to cold led to attacks of periodic paralysis in some of his cases Minor instances of periodic paralysis should be sought for in many metabolic upsets where asthenia, stiffness, and aching of muscles are a transitory complaint.

Harvey has recently pointed out that both hypopotassemia, as shown above, and hyperpotassemia, as met with in some cases of renal failure, may cause muscular weakness and dysfunction, and that a guide to proper therapy may lie in the T wave change of the electrocardiogram which is a sensitive indicator of the serum potassium level (80a)

Lathyrism This strange disease results from ingestion of too large or unbalanced amounts of the lathyrus pea (chickpea) Epidemics have naturally occurred in famine times. Onset is usually sudden, with pain in the lumbar region and weakness and spasticity of the lower limbs Sometimes the onset is more gradual. Spastic paraplegia is the result, with muscular cramps and little or no sensory disability. Denny-Brown (43) has reviewed the literature. Stockman (169) produced paralytic effects in monkeys with aqueous extracts of Lathyrus satirus It has recently been reported that a

phenomenal increase in serum alkaline phosphatase occurs in human lathyrism (154). This has not been confirmed in animal experiments (33). The entire situation should be surveyed again in the light of previous work and with a view to establishing a deficiency or food-toxic mechanism.

Tick Paralysis. This amazing condition results in rapid motor paralysis much in the vein of Landry's ascending paralysis. It is widespread throughout the world, but in Canada and the United States is mostly limited to the western mountain parts. In these regions the wood tick (Dermacentor andersoni stiles) is responsible. A patient, perfectly well one day, may the next day complain of weakness of the lower limbs and shortly develop weakness of the arms, difficulty in swallowing, and even respiratory trouble. There is little pyrexia, but some increase in pulse rate. The condition is usually confined to the motor system. The time from beginning of symptoms to complete paralysis, or even death, may be as little as 2 to 5 days Recovery occurs rapidly if the tick or its head is removed from its burrowing place under the skin of the patient. In children this is often around the neck or hairline.

The powerful toxin (or whatever it is) seems to be generated in the ovaries of some fast-feeding ticks, and is injected parenterally into the body of the victim. Both animals and man may be affected This should be an important research tool (118,151)

The reversibility, upon removal of the tick, is almost as dramatic as that obtained by administering potassium in periodic paralysis, neostigmine in myasthenia gravis, or quinine in myotonia. This suggests that paralysis may be due to action of the toxin upon the muscles directly or upon the acetylcholine system at the neuromyal junction. No paralysis results from the injection of blood of affected animals or of the intestinal contents of ticks taken from paralyzed animals (8). Injection of extracts of suitable ground up tick eggs, however, does produce typical tick paralysis in the dog (75). Normal muscle response to faradic stimulation of an exposed peripheral nerve in a paralyzed dog was obtained by Ross (153). He concluded that the paralytic agent acts centrally The animals were anesthetized in his experiments, and there is the possibility that the anesthetic interfered with the action of the toxin Similarity of tick paralysis to conine poisoning has been noted (50).

Further investigation of this disease, using alkaloids such as strychnine, curare, or quinine, and other substances known to affect the neuromuscular apparatus such as eserine, procaine, DFP., acetylcholine and neostigmine at the time of onset of symptoms, together with muscle biopsies and electromyography, may throw light upon unexplored aspects of tick paralysis and may help to explain the lower motor neuron involvement.

Jake Paralysis (Triorthocresyl Phosphate Neuropathy). Among the exogenous factors capable of producing a clinical picture of polyneuritis in which motor neuron involvement predominates, mention should be made of Jake Paralysis. Triorthocresyl phosphate, when used as an adulterant in a Jamaica ginger alcoholic beverage or if present in a certain toxic abortifacient oil (apiol), has produced symptoms which closely resemble amyotrophic lateral sclerosis. There may be weakness, atrophy, and even paralysis of the small muscles of both hands and feet, with loss of reflexes. Fasciculations, contractures and hyperalgesia have also been noted (73, 74).

The toxic effect on the entire lower motor neuron was noted by Aring (5) Lesions in the affected muscles included spotty loss of fibers with connective tissue and fat replacement, and arteriolar and capillary hyperplasia The lower motor neuron showed evidence of hypertrophic neuritis, with swelling and chromatolysis of anterior horn cells In the cord, the pyramidal fibers were chiefly involved The neurologic symptoms only appear in humans about 7 to 14 days after ingestion of the poison Aring suggests that reduced blood supply, due to the arterial hyperplasia, may be the cause of the neurologic lesions and that the vascular changes develop during the latent period

DDT (Dichlorodiphenyltrichloroethane) Poisoning. There is evidence that this substance acts chiefly upon the neuromuscular apparatus, but also upon higher parts of the nervous system

Persistent, involuntary tremors and twitching of muscles, stiffness and occasional epileptoid convulsions, and clonic contractions have been observed both in man (165) and in animals (156) Symptoms are reversible if doses are small, but may go on to flaccid or spastic paralysis and death A central site of action has been suspected because of electroencephalographic evidence of marked in-

crease in cortical activity in the cat and monkey (37), and demonstrable diffuse damage to ganglion cells of the brain with vacuolar degeneration or pyknosis in the cat (145).

Acting on Yeager and Munson's demonstration (188) that the repetitive discharge of nerve impulses into the muscle of the roach exposed to DDT may be due to a peripheral effect of this drug at the neuromyal junction, Carey *et al.* (30) investigated the morphologic appearance of nerve endings, neurosomes and fiber types in the voluntary muscles of chameleons and rats injected with DDT. The intermittent and incoordinate tremors obtained resembled both fibrillations and fasciculations of denervated muscle. Microscopically, the acute atrophy and variation in distribution of gold-staining granules, either at the end-plate or through the muscle fiber, was similar to that observed after experimental denervation

Spider and Snake Venom Poisoning. (1) Spider Bite. Certain spiders elaborate venom with predominantly neuromuscular effects and some species, e g , Latrodectus, may be very deadly. Important species of the genus Latrodectus are the "red-backed" spider of the antipodes (L hasseltii) and the "black widow" of California and the Southern United States (L mactans)

Bogen (19) reported 150 cases of spider bite, most of them in males. The majority were bitten on the genitalia or adjacent parts while using outdoor privies. At the instant of the bite there is a sharp, stinging sensation which is acutely painful but transient, 15 to 45 minutes later, acute pain is felt in nearby regions, which spreads centrifugally and is apparently due to painful muscle spasm. It is most severe in the abdomen and legs, but it also spreads to the back, shoulders, and arms Recurrent cramps and muscular twitchings are also seen The thighs are usually flexed on the abdomen and the legs are flexed on the thighs, the arms folded on the chest. Other, more variable symptoms are seizures, vomiting, shock, urticaria, and urinary retention. The most prominent physical finding is a boardlike rigidity of the abdomen and spasm of muscle groups in the extremities The acute symptoms last 24 to 48 hours, but some cramps may persist for 1 or 2 weeks.

There is increasing evidence that drugs acting on the neuromuscular system are most effective in combatting this spasm. Bell and Boone (12) have described a patient who was dramatically re-

lieved within 1 hour by intramuscular injection of neostigmine after intravenous administration of calcium gluconate and sedatives had failed. Davis (40) recommends curare as the treatment of choice, but states that extreme pain and muscle spasm may be relieved in 5 minutes by the intramuscular or intravenous use of a sterile 25 per cent solution of magnesium sulfate.

(2) *Snake Bite.* Certain snake venoms, especially those from the cobra group, have a powerful curare-like action in addition to hemolytic and anticoagulin properties. These venoms kill through their paralyzing effects on the neuromuscular apparatus peripherally. First weakness of the limbs, oculomotor palsies, and ataxic gait appear. Dysarthria and nasal voice may then develop and swallowing becomes difficult Death ensues from peripheral respiratory weakness (58). It would seem desirable to try the effect of neostigmine in these cases.

Tetanus. When injected into or elaborated within an infected muscle, tetanus toxin causes local tetanic changes in the muscle After a latent period it also reaches the spinal cord, and more widespread and complex spasms result. Evidence still favors the view that the toxin spreads centripetally up the motor nerve fibers and thus reaches and stimulates the anterior horn cells The tetanus is thus at first a muscular phenomenon and later a neuromuscular one Roofe (152) believes that the neurofibrillae of the axis cylinder are the agents responsible for toxin transportation to the anterior horn cells, and he has determined the rate of progression to be 3 35 mm per hour

Harvey (78) has made electromyographic and pharmacologic studies on muscle poisoned by local injection of tetanus toxin. The development of stiffness in the affected muscle is associated with a constant high level of repetitive activity in many fibers. This continues in spite of nerve section, but ceases when the nerve degenerates. It is stopped by curarization. Electrically, the condition is not unlike myotonia or the fibrillary activity in denervated muscle

Gammon, Harvey, and Masland (66) contrast these three conditions One of the most important differences is in the tone of the affected muscle The denervated muscle is flaccid; myotonic muscle is stiff and shortened during the aftercontraction and the tetanus-poisoned muscle exhibits a boardlike rigidity. The fibrillation of de-

nervation has been attributed to stimulation by acetylcholine; on the other hand, curare does not stop it nor does eserine potentiate it much The fibrillary activity of tetanus-poisoned muscle is promptly inhibited by small doses of curare It has been suggested that in tetanus poisoning the nerve endings release abnormal amounts of acetylcholine, and that through lack of cholinesterase in the tissue the acetylcholine persists and causes continued stimulation of the muscle (66).

Since West's (185, 186) comprehensive experiments, a number of authors (26, 183) have testified to the beneficial effects of curare or curare substitutes in suppressing the spasm of tetanus. At first this was a hazardous procedure, but with the development of purer alkaloids of standardized potency and with slow-absorbing, repository preparations, such treatment has become feasible and may replace nervous system depressants such as avertin. Much of the cause of tetanic spasm stems from the central nervous system, however, and this may explain the beneficial effect of general anesthetics and of myanesin

Botulism This disorder is produced by ingestion of the toxin of Clostridium botulinum which grows in spoiled food Gastrointestinal disturbances occur in only about one-third of cases Neuromuscular symptoms are predominant and develop after a latent period of several hours to 4 days The picture resembles myasthenia gravis There are visual disturbances, ptosis, diplopia, difficulty in speaking, chewing and swallowing, and generalized fatigability on effort. Limb and neck muscles show weakness in 80 per cent of the cases Dysphagia and respiratory paralysis may occur. There is no sensory disturbance nor change in tendon reflexes Temperature, blood pressure, and function of other organs are unaffected Terminal coma or convulsions may occur, but in most cases consciousness is unimpaired.

The paralytic effect of the toxin upon the muscle nerve endings has been demonstrated by Dickson and Shevky (48)

Electromyographic records of the peripheral action of the toxin in guinea pigs and rats have been made by Masland (119) Local paralysis was produced by intramuscular injection of a minute amount of the toxin The electromyogram of the intoxicated muscle showed characteristic features which distinguished it from the nor-

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mal, and also from curarized muscle. It showed that the initial response to nerve stimulation was reduced to as little as 10 per cent of the normal response. There was never any evidence of the rapid fall in potential so characteristic of curarization The records resembled those obtained in experimental tetany in animals suffering from dietary hypocalcemia The administration of neostigmine scarcely affected the response.

Burgen *et al.* (25a) have illuminated the problem of botulism by fundamental work. They have shown that the paralysis produced by botulinum toxin on the rat phrenic nerve-diaphragm preparation is due to neuromuscular block. Conduction in the nerve is unaffected, and the muscle responds normally to direct stimulation. The neuromuscular block differs from that of curare in that the motor end-plates remain sensitive to acetylcholine and in that *the output of acetylcholine on motor nerve stimulation is greatly reduced.* Ambache (3a) has shown that muscle into which botulinum toxin has been injected locally is completely unresponsive to motor nerve stimulation, but responds to close arterial injection of A Ch. It would be profitable if this type of experimentation could be applied to the study of various neuromuscular disorders in man.

These experiments indicate that the peripheral action of botulinum toxin differs from that of curare This is borne out by clinical experience, since eserine and similar drugs have proved ineffective in botulism despite its apparent resemblance to myasthenia gravis (161)

Summary

The above review illustrates the wide variety of mechanisms responsible for neuromuscular disorders. Some mechanisms are clear and others are dim upon the horizon. Certainly there is reason to believe that each and every one of these disasters will find explanation in the future.

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Use of Sodium Depletion in Therapy

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Introduction

It is not unusual for physicians to be confronted by a series of reports that a therapeutic agent or procedure is remarkably effective in a wide variety of unrelated disorders, and by other articles in which each of these claims is meticulously disproved. The normal response is disbelief in the optimistic reports, perhaps with the reservation that where there is so much smoke there must be some fire Foxglove was disdained by the profession, although it had been used with success for centuries in England by quacks, empirics, and wise women. Withering's report of its value in cardiac dropsy was accompanied by case histories suggesting that it cured scarlatina, and soon by Beddoe's claim that it was as specific for phthisis as cinchona was for ague No wonder most nineteenth century doctors doubted its value and used it in inadequate doses The therapeutic value of sodium depletion has had a similar fate.

At the turn of the century, salt depletion was recommended for the control of edema by Widal and Le Mierre (1) and for hypertension by Ambard and Beaujard (2) In 1926, Sauerbruch *et al.* (3) enthusiastically proclaimed its merits for hastening the control of tuberculosis, and within another decade it was described as invaluable in arthritis (4), and also in Ménière's syndrome, epilepsy, and narcolepsy. The last ten years have seen a rise in enthusiasm for its use in heart failure and in hypertension But, in the meantime, American authors reported the salt-poor diet as valueless in tuberculosis and in arthritis Little was written in favor of its use in hypertension, or even in heart failure, between 1925 and 1944 Allen and Sherrill (5), who used it with satisfactory results in their hypertensive patients, failed to convince physicians that the

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regime was effective. However, Volhard and others in Germany continued to write on low sodium intake for hypertension, and the increasingly rigid diets eventually led to Zondek's diet based on rice. This food was chosen because of the low incidence of hypertension in countries whose chief cereal and source of calories is rice.

It is an interesting fact that salt-depletion therapy fell out of fashion in America just at the time the mercurial diuretics were introduced. These made it easier to control edema in patients with cardiac disease or cirrhosis without rigid salt restriction. They also made it possible to deplete hypertensive patients faster when salt was restricted, but they were not used for this purpose until after the rice diet had been introduced to America in 1944

Kempner, who chose rice and fruit as the sole natural components of a regime for hypertensive patients, based his diet on the supposition that all hypertension is of renal origin and that protein, salt, and perhaps even fat would add to the metabolic load of supposedly under-oxygenated kidney (6–9). He was fortunate in beginning his clinical work in the part of the United States where rice is widely used and appetizingly prepared and he has had unequalled success in maintaining patients on a drastically limited diet for periods of years Up to 1948 his reports appeared only in the journals of local medical societies, but the excellent results he obtained were soon widely known. The regime itself did not gain wide acceptance among physicians However, patients from every part of the United States, many of them physicians, returned from Durham and often astonished their previous medical attendants by their low arterial pressures, good health, and zealous adherence to a rigid regime.

At the same time, however, there were reports from careful and experienced workers that in controlled, hospitalized patients sodium depletion was not especially effective in reducing blood pressure or relieving the symptoms of hypertension (10, 11) It was also reported that after 90 days on such a diet a patient might still be in negative nitrogen balance, while in the early weeks the negative balance was more than 3 Gm per day, representing a loss of over 18 Gm of body protein daily (12) That the low-sodium diet, even in the low-protein modification of the rice regime, could precipitate uremia in patients with poor renal reabsorption of sodium was

apparent in several reports which noted fatalities (13, 14). Such findings, reported in journals with wide circulation, led many physicians to regard with incredulity the optimistic reports of Kempner. However, one group who tried a diet with 200 mg. sodium, 70 Gm. protein, and liberal fluid intake, reported results in 45 ambulatory hypertensive patients which compared more than favorably with those obtained by sympathectomy on similar patients in the same clinic (15). Since these results also approximate those of Kempner, the necessity for an extremely low protein regime appeared questionable.

While experience has proved that in disorders such as diarrhea or diabetes sodium depletion always causes dehydration, and that this is also the case in the anasarcous patient, it is worth emphasizing that in the latter sodium deprivation may lead to a significant fall in serum sodium, below the normal range, before actual dehydration is attained. The mechanisms which initiate maximum reabsorption of sodium by the renal tube also evoke maximal water reabsorption. The latter occurs very promptly, on effort or even on assuming the erect posture, and it operates even after sodium depletion has robbed the body of considerable electrolyte Sodium excretion normally equals sodium ingestion, with a lag of some days in establishing equilibrium when intake is raised or lowered. Sodium excretion in sweat and urine falls markedly when sodium intake is restricted or sweating is profuse enough to deplete sodium In patients with continuing heart failure or cirrhosis treated by salt restriction and mercurial diuretics, urinary excretion of sodium on a daily intake of 200 to 500 mg. may fall below 10 mg. per day, and the patient will go into positive sodium balance after diuretics are discontinued If serum sodium is reduced by diet at a time when the mechanism for fluid retention is being powerfully stimulated by severe heart failure, uremia develops, edema becomes stationary, and salt administration is essential to avoid death. Fortunately, in most cardiac patients, the heart condition improves and uremia decreases on sodium depletion

It is generally conceded that dehydration can only be effected if sodium is depleted, and that dehydration is of value in epilepsy, Ménière's syndrome, narcolepsy, and in all the states in which there

is obvious edema of the limbs or lungs. Where specific therapy, such as anticonvulsive drugs for epilepsy, benzedrine for narcolepsy, digitalis and mercurial diuretics for heart disease, or high protein diets for cirrhosis, is effective, the low sodium regime is obviously discontinued early, or perhaps never instituted Thus, even in conditions for which its efficacy is not disputed, the low sodium diet is by no means widely used or maintained as long as the underlying disorder persists. No really agreeable low-sodium diet has been prepared, and the social restrictions attendant on all rigid diets are galling even to those who eat to live, rather than live to eat.

Rationale of Sodium Restriction in Hypertension

Hypertension is a disturbance in the level of one of the physiologic constants, and in this respect must always be compared with fever, a disturbance in level at which body temperature is maintained in the isothermic vertebrates While there may not be as many causes of hypertension as of fever, there is no reason for believing that the primary cause is the same in all cases In animals, hypertension has been evoked by repeated audiogenic insults (16, 17), by various procedures which reduced renal pulse pressure, often with no decrease in renal venous oxygen tension (*i.e.*, with no ischemic anoxia), by progressive depressor denervation, by ligation of carotid and vertebral arteries, and by treatment with desoxycorticosterone and salt. In man, it has been relieved by removal of adrenomedullary and of adrenocortical tumors. In some young hypertensive patients with unilateral chronic renal lesions, it has been cured by removal of the diseased kidney. Many hypertensive patients show familial over-reaction of the blood pressure to emotional states In such cases, the only renal disturbance in the early years of the disorder is a high vasomotor tone in the kidney. This can be abolished by induced fever, or by pyrogens even if fever is prevented by aminopyrin. Renal ischemia is not the cause of most cases of hypertension

There is accumulating evidence that in most cases of hypertension a number of factors are acting together to raise the pressure—that the response to various initial stimuli involves many hormonal and vasomotor mechanisms supplementing each other Thus, epinephrine from a tumor of the adrenal medulla acts through the hypo-

thalamus and pituitary to increase adrenocortical secretion. Salt metabolism may be secondarily disturbed. Hypertensive patients of various types show a uniformly rapid fall in sodium excretion when shifted to salt-free diets. They also show pressor responses to desoxycorticosterone which, like the reduced sodium excretion just mentioned, are far more abrupt and striking than those of normal individuals subjected to the same procedure. Most cases of hypertension, renal or otherwise, show some fall in blood pressure when given full doses of sympathicolytic drugs, such as *tetra-ethylammonium chloride* But even labile, emotionally evoked hypertension may show elevated pressure levels, as compared with normal controls, under full doses of such drugs. Whether the active humoral element is renal, adrenocortical, pituitary, or from the central nervous system is not known

Since hypertension results from various causes, and involves several mechanisms operating at different levels in any one case, it is not surprising that neither sympathectomy nor severe sodium depletion has been found, even by enthusiastic proponents, to bring pressure back to normal in half of any series of cases From 20 to 30 per cent show no appreciable change in pressure after either method Both procedures, in combination, have failed to restore normal pressures in some cases, while either alone may appear to be curative in 10 to 30 per cent Patients in whom brief sodium restriction has lowered the basal morning pressure often show unchanged casual pressure levels throughout the day in the hospital Basal, but not casual pressures, may go up when salt is added to the diets of these patients In the same way, salt restriction may augment the pressure-lowering effect of sympathicolytic agents in some cases, and have almost no effect in others These variations in the nature of the hypertensive state must be clearly understood Hypertension is not *a disease, like pneumococcic pneumonia, but a disturbance like fever* or like muscular spasticity It would be unreasonable to expect a single specific therapy, in view of the variations in etiology

Biologic Aspects of Sodium Intake and Its Relation to *Blood Pressure Level*

When normotensive adults are given enough sodium chloride to double or triple their usual intake, there is little rise in their blood pressures Even when desoxycorticosterone is given with a liberal

salt intake, the rise in blood pressure only develops after a week or two. In either experiment ankle edema and puffiness of the eyelids are apt to be apparent before the pressure rises. However, when McQuarrie *et al.* (18) gave large daily doses of salt to diabetic normotensive children, they observed a rise in pressure in a short time in almost every case. Control studies on children without diabetes have not been made In short-term experiments with animals, high salt intake has little effect on blood pressure unless there are renal dysfunctions or unless large doses of desoxycorticosterone are given concomitantly. Apparently, salt intake at levels above those usual in the mammalian diet can be tolerated without the early development of hypertension unless endocrine balance, or perhaps hypothalamic function, have been disturbed

Sodium plethora does occur when patients with Addison's disease are overtreated with desoxycorticosterone and salt. This may precipitate heart failure, and in rare instances it has precipitated rheumatoid arthritis. Real hypertension has been evoked in many cases, but most of these were known to have been hypertensive before the adrenal insufficiency developed That severe hypertension can be caused by sodium plethora alone, or that oversecretion of a desoxycortocosteroid is present in any significant proportion of hypertensive individuals has yet to be established. Conn *et al.* (19, 20) observed that the electrolyte content of sweat is low when such a steroid is given to human subjects, or when autogenous secretion is at a high level, as in Cushing's syndrome or in adaptation to a hot environment Hence, electrolyte content of sweat may be used to detect overactivity of this part of the endocrine system and may serve to demonstrate or rule out this factor in cases of hypertension

Students of hypertension have been struck by the fact that hypertension apparently is rare and the basal and casual levels of blood pressure are lower in undernourished populations in the tropics and in the Orient than in Europeans living in the tropics, or in Negroes transplanted to the New World, including such tropical regions as Panama This has been ascribed by some to different philosophies of living—to the renunciation and acceptance of life by the native populations of the tropics and the Orient, or to the struggle for escape or success in the West Others have thought that the genetic differences—differences in endocrine or nervous response to stress

—were responsible for any real differences. This has not been disproved for the Orientals as thoroughly as in the case of Negroes, who have very different blood pressure levels and disorders in Africa and in Panama The role of diet in these alleged racial or regional differences in blood pressure has often been mentioned, but the relative importance of low sodium and low protein content is disputed

The sodium content of the diet of all mammals, except those which live on salt water fish or shellfish, is relatively low. The herbivores probably do not ingest more than 100 mg. sodium per 1,000 calories Even less is obtained by those birds and mammals, including the great apes, which live largely on fruit, nuts, and seeds, including grasses and grains. Among the root-eating or omnivorous rodents, swine, and bears, the sodium intake is closer to 200 mg. per 1,000 calories, while the carnivores obtain 300 or more mg. per 1,000 calories The walrus, living on shellfish, takes in 6,000 mg of sodium per 1,000 calories and never has a drink of fresh water! Most marine mammals, living largely on fish but swallowing some sea water, take in 400 mg or more per 1,000 calories. An American laborer getting 3,000 calories a day in restaurants probably takes in 8 to 12 Gm of sodium, as much per kilogram per day as the walrus, 25 times as much per kilogram as the lion, and 200 times as much as his closest mammalian kin—the gorilla, orangutan, and chimpanzee. Normal American diets provide 50 to 100 times as much salt as the anthropoid diet of fruit and nuts

Because of the need for sodium in the internal environment and the low sodium content of most foods, animals and primitive people may travel long distances to salt deposits The animals can only store sodium in extracellular fluid and possibly as proteinates in the skeletal and conective tissues Sodium content of cartilage and bone, in mols, is 2 to 3 times as great as chloride, whereas elsewhere the sodium is only 10 to 30 per cent more abundant The difference presumably is proteinate Actual sodium storage, as affected by diet or depletion, has not been carefully studied But the sodium the herbivores get at a salt lick does not produce visible edema, and seems to be drawn upon for many weeks Sodium conservation is excellent in all mammals, and those which live in the tropics may have almost electrolyte-free sweat In men acclimated to hot environments, electrolyte content of the sweat is also low. In all men, so-

dium in sweat rises with decreasing adrenal function and is lowered by desoxycorticosterone (20).

The diet of primitive tribes in tropical regions with heavy rainfall is extremely low in sodium, which has been leached from the soil They often live largely on cassava and sago, from which sodium and protein are leached in the course of their preparation as food Sago, arrowroot, and manioc, as eaten, contain less than 15 mg sodium per 1,000 calories In the rain forests, salt deposits are nonexistent, and salt is an expensive import, carried hundreds of miles on human backs and usually heavily taxed as well. The sodium intake of natives of the rain forests distant from the sea may be as low as 45 mg per day. The protein intake is also very low Manioc, sweet potatoes, and bananas provide their staple fare with an average protein content of 5 or 6 Gm per 1,000 calories; rice provides over 20 Gm per 1,000 calories Hence, any effect that these diets may have can not be ascribed to low sodium intake alone The sodium intake of most Asiatic and tropical peoples, eating little meat, rarely exceeds 200 mg per day The sodium levels of our "salt-free" and rice diets are thus equal to those of the normal diets of the majority of the world's human population, and are much higher than those of millions of natives of the tropics Our sodium losses in sweat are lower than those of most of these people, who go through life just barely out of sodium depletion or actually below the minimal level for health

It is unfortunate that we do not have dependable data on the blood pressure levels and the incidence of hypertension at various decades of life among peoples living on various levels of salt intake, and none on what happens to groups of people whose salt intakes are suddenly raised from these usual low levels to those of well-fed Americans All the evidence at hand indicates that blood pressure levels are low in the people living on protein and salt intakes similar to those of patients on our lowest protein and salt diets Since low protein accompanies low salt intake in nearly all of these native diets, this experience does not help in evaluating the importance of salt and of protein individually The biologic evidence merely indicates that low-sodium, low-protein diets suffice to permit vigorous muscular activity and reproduction, that they are the usual diets of millions of men, and the invariable diets of all the anthropoids. The salt-poor diet and the rice diet may be unappetizing, but they are physiologic. When any one suggests such a diet for treatment of any

of the disorders encountered in Western civilization he can base his reasoning on the old slogan "Back to Nature." But such a basis is circumstantial and incomplete; reasonable men may remain skeptical

EVIDENCE IN ANIMAL AND CLINICAL EXPERIMENTS

At the outset, it must be noted that the reasons given by Kempner for using a low-protein, low-salt, low-fat diet lack experimental support. Even granting the unproved thesis that all hypertension is due to renal pressor hormones, there is no evidence that renal metabolism is significantly raised by increasing the salt and fat content of the diet, and even when the osmotic work done in excreting urea is increased fifty fold, renal oxygen consumption rises less than 25 per cent (21). There is no demonstrable increase in renal oxygen use when rats are changed from a protein-free to a 74 per cent protein diet (22), but since renal hypertrophy takes place some change in renal metabolism must occur. The extra renal work needed to concentrate urea or salt can be obviated by drinking more water, which most patients would greatly prefer to changing their diets. However, the aromatic acids derived from protein are actively secreted, and the metabolic effects of increased intake of protein on this phase of renal metabolism cannot be abolished by increased fluid intake. Whether there is any relationship between the level of aromatic acid excretion and renal work or blood pressure regulation is not yet known.

It is clear, from studies of renal vein blood from hypertensive patients (23) and from animals with constricted renal arteries (24) or with other renal lesions which evoke hypertension, that renal anoxia is not present in renal hypertension or benign hypertension The renal vein blood normally has a relatively high oxygen content, and renal metabolism varies with renal blood flow (25) As a consequence, even when flow is 30 per cent of normal, the blood coming from the kidney has a far higher oxygen content than that coming normally from the liver The metabolism of anaerobic kidney slices, studied in the Warburg apparatus by Kempner and others, seems irrelevant to the problem of hypertension

In every series of rats or dogs in which low sodium diet has been tested, it has proved effective either in lowering established renal hypertension, or preventing a rise which occurred in control animals with renal lesions of similar character. One group of rats had nephro-

dium in sweat rises with decreasing adrenal function and is lowered by desoxycorticosterone (20).

The diet of primitive tribes in tropical regions with heavy rainfall is extremely low in sodium, which has been leached from the soil They often live largely on cassava and sago, from which sodium and protein are leached in the course of their preparation as food Sago, arrowroot, and manioc, as eaten, contain less than 15 mg. sodium per 1,000 calories In the rain forests, salt deposits are nonexistent, and salt is an expensive import, carried hundreds of miles on human backs and usually heavily taxed as well. The sodium intake of natives of the rain forests distant from the sea may be as low as 45 mg per day. The protein intake is also very low Manioc, sweet potatoes, and bananas provide their staple fare with an average protein content of 5 or 6 Gm. per 1,000 calories; rice provides over 20 Gm. per 1,000 calories Hence, any effect that these diets may have can not be ascribed to low sodium intake alone The sodium intake of most Asiatic and tropical peoples, eating little meat, rarely exceeds 200 mg per day The sodium levels of our "salt-free" and rice diets are thus equal to those of the normal diets of the majority of the world's human population, and are much higher than those of millions of natives of the tropics Our sodium losses in sweat are lower than those of most of these people, who go through life just barely out of sodium depletion or actually below the minimal level for health

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Addis, and Kempner include, with the diet, the effects of strong personalities and of celebrated clinics on patients, most of whom have unusual faith and confidence in the therapy.

Some have dismissed the clinical reports, summarized in Table I, and several series of less than 10 patients which show greater variability in results, as showing only the variations in the natural history of a labile disorder. In addition to these reports, there have been two studies which included attempts to determine the mechanism through which sodium depletion might operate. In one, the response to sympathicolytic inhibition of the vasoconstrictor tone

TABLE I

Summary of Reports on Treatment of Hypertension with Salt-Free Diet

Author and ref no	Diet		Number of cases	Per cent of cases showing significant fall in pressure
	Sodium/day mg	Protein/day Gm		
Kempner (29)				
Over 35 days on diet	< 200	< 25	305	70
Less than 35 days on diet			195	51
Bryant and Blecha (15)	200	70	45	58
Flipse and Flipse (30)	< 200	< 25	32	59
Viersma (31)	400	60	14	36

was tested before the regime, and during sodium depletion (32) Of the 12 patients tested on a diet with 250 mg. sodium per day and "adequate protein" for 7 to 21 days, only 1 showed a significant fall in diastolic pressure Mercurial diuretics were given to hasten salt loss, but serum sodium values were rarely lowered. In 5 of the patients the fall in pressure evoked by giving 400 mg. of tetra-ethyl-ammonium chloride was significantly increased by sodium depletion In 1 case, the fall was greater in the periods in which serum sodium fell below 131 mEq than at times when it was higher In 7 cases, no fall in serum sodium or in the blood pressure "floor" during sympathicolysis was observed It was concluded that while salt depletion

toxic (Masugi) nephritis (26), one group had perinephritis (27). In the former group it was shown that desoxycorticosterone aggravated the renal lesion and hypertensive state if given with salt but had no effect on the condition if the rats were on sodium-free diets (26). In the latter group it was shown that potassium chloride had no effect on blood pressure, while sodium chloride, added to the salt-free diet, promptly raised the blood pressure (27). In these experiments the diets were not particularly low in protein. On the Kempner diet, 11 dogs, hypertensive for 2 to 4 years, had an average fall in mean arterial pressure from 182 mm. Hg. to 138 mm. in 8 weeks (28). All lost weight and failed to take the daily offered 900 calories "One dog refused the diet entirely and died before any significant observations were made " Plasma protein was maintained at a constant level (6.2 Gm./100 cc.).

These experiments establish a valid experimental basis for the use of sodium depletion in renal hypertension with minimal urea retention They indicate that a low protein diet is not always essential to obtain striking effects The value of protein or fat restriction, and the effects of salt depletion in other types of experimental hypersion, remain to be determined

Since 1940, clinical investigation in this field has been very active, and the results offer a certain uniformity, if allowance is made for the effect of psychotherapeutic influences. In general, fewer and less dramatic falls in pressure and greater persistence of symptoms are characteristic of the series with rigidly controlled hospitalized patients, in the hands of skeptical but honest observers Higher percentages of falls to normal levels, relief of symptoms, and improvements in cardiac and retinal lesions occur in cases with minimal hospitalization in the hands of enthusiastic or optimistic but honest observers Psychogenic factors apparently play a role in many cases of hypertension Therefore the results of any form of treatment taken by enthusiastic, hopeful patients, who have chosen to make a pilgrimage to a noted healer, forsaken friends, family, and occupation, and who are surrounded by others satisfied with the regime, cannot be compared with results in patients going to a general hospital, and being put on a disagreeable regime, different from patients in adjacent beds, in order to satisfy the curiosity of the clinical staff. It seems clear to us that the results of Allen, Volhard,

supreme faith in the rice diet is put on a more liberal diet by some well-meaning physician, he may easily develop anxiety and even a sense of guilt, just as would a Mormon, a Seventh Day Adventist, an Orthodox Jew, a Catholic, or a Moslem who was led by skeptics to violate the dietary rules of his religion. Perhaps only from animal experiments can convincing evidence on many of these points be obtained, and even here psychogenic influences of severe regimes can not be ignored However, failure of blood pressure to return to hypertensive levels in dogs successfully treated with the rice regime, if given a low-salt regime rich in protein and fat, would completely negate Kempner's inspired selection of rice and fruit after noting the metabolic effects of anoxia in Warburg tests on kidney slices

The role of the salt and protein content of the diet receives some further illumination from the observation that most, but not all, hypertensive individuals who suffer Addisonian crises of adrenal insufficiency have a fall in blood pressure to normal levels, and that hypertension is rare, but not absent, in cirrhotic patients who have long been on low protein diets and are in severe states of protein deficiency. When one has seen such a cirrhotic patient, who has been robbed of protein and sodium by repeated paracentesis, by salt restriction, and by mercurial diuretics but who still maintains a blood pressure well above the 150/90 level, one realizes that hypertension will not always be relieved by severe sodium and protein depletion. When one sees a patient with Addison's disease whose usual levels of blood pressure on maintenance doses of desoxycorticosterone is 250/130, come in with adrenal insufficiency, hemoconcentration, and vomiting, but with the pressure still 180/100, one realizes that adrenalectomy and negative sodium balance will not always restore normal pressure levels to the hypertensive patient One such patient has been followed for years, and has no evidence of pheochromocytoma Hypertension, a disorder due to various causes, probably will not respond to a single specific remedy Not more than 80 per cent show an appreciable effect of maximal psychotherapeutic influence plus rigid restriction of salt, protein, fat, and such common refreshments as tea, coffee, and chocolate that is, the rice-fruit regime In many of these the blood pressure continues at abnormally high levels. The response is no better in benign than in renal hypertension, and is less brilliant in human than in experimental renal hypertension In

might lower the humoral component in hypertension, rise in vasomotor tone compensated for this and pressure was held at a fairly constant level

In the other study, 6 patients with hypertension were kept on diets with 70 to 80 Gm. of protein, and the sodium intake kept at the 250 to 350 mg. level for 2 weeks (33). In control periods, 4 and 15 Gm. sodium chloride were given Average pressures were 170/108 mm Hg after a period on 4 Gm of salt, 155/95 at the end of salt restriction, and 170/108 after being on 15 Gm of salt. When desoxycorticosterone was given with the 4 Gm. of salt, pressures rose to 185/114 When desoxycorticosterone was given on a low salt regime, pressure fell again to 158/98. While the periods of observation were brief, these results seem significant, and confirm the observations in animals that desoxycorticosterone has no pressor effect during sodium depletion under conditions in which it is otherwise effective. In this study, basal morning pressures fell, while casual pressures were unaffected by salt restriction These investigators arrived at the conclusion that salt deprivation leaves unaltered or actually increases the "neurogenic" element in human hypertension, but it may reduce a humoral or hormonal component.

The results of the experiments in which sympathetic outflow was blocked before and during salt depletion, and the observation that during sodium depletion morning basal pressure levels fall more than "casual" or random pressures, bears out the importance of neurogenic effects in determining the pressure level A sodium-free regime, with nothing to change the mood of the patient from anxiety and frustration to faith and renunciation has a demonstrable effect on few cases of established hypertension as compared with a regime which at the same time causes a favorable change of mood Since there has been no series showing purely psychotherapeutic results comparable to the best dietary series, a genuine therapeutic effect of salt restriction in many hypertensive patients seems established

Comparison of results in patients on the rice diet and on diets low in sodium but much richer in protein and fat has not established the need for protein and fat restriction Here again it will be difficult to evaluate the psychogenic component In many patients, resistance to and frustration resulting from the rice diet is far greater than with a more liberal regime On the other hand, when a patient with

When acid resins with an uptake of 1 per cent or less are taken by mouth, after being washed with distilled water, they are tasteless and cause no distress Acid resins with a capacity of 6 per cent or more take base out of the fluid so fast that some burning is felt in the mouth and there may be real distress in the esophagus and the abdomen For this reason high capacity resins would have to be used as potassium or ammonium compounds. A blend of acid, calcium, potassium, and ammonium resins might be used. This would not deplete the body store of potassium or calcium and would be free from unpleasant side effects.

Only one clinical study of the use of resin has been completed (35) A resin with a maximum capacity of 3 9 mEq per gram (9%) was fed, in capsules, in daily dosage up to 70 Gm, to one hypertensive patient over periods of weeks, and to 2 edematous cardiac patients and to one with cirrhosis All were on diets with a fixed electrolyte content; usually the sodium chloride intake was 3 to 6 Gm per day. A surprising fact was that although, from equivalent mixtures, *in vitro* uptake of calcium was greater than that of sodium or potassium, in the patients no fall in urinary or rise in fecal calcium was observed. In the case most carefully followed, urinary sodium loss fell from 109 to 10 mEq per day, potassium loss from 80 mEq to 8 Fecal sodium loss rose from 3 to 60 mEq, potassium loss rose from 5 to 60 mEq per day. Ammonium excretion in urine rose from 25 to 160 mEq. per day. In all cases, the carbon dioxide combining power of plasma fell, in one from 55 to 19 volume per cent

The three edematous patients had required mercurial diuretics at 2 to 10 day intervals before starting on resin. Weight fell without resort to mercury while resin was being taken One lost 7 5 Kg in 13 days, the other 4 Kg in 12 days Serum potassium fell only in the cirrhotic patient, in whom it was low before resin was given No toxic symptoms ascribable to the resin were observed, but the hazard of fecal impaction was recognized. It was concluded that this resin, given in doses capable of 150 mEq maximal sodium uptake daily, lowered urinary sodium excretion from an initial 100 mEq level to 50, at a dose equivalent to 250 mEq uptake, sodium excretion fell to 8 mEq Such a regime also lowered the sodium in the body and decreased edema No study of the effect of resin on a series of hypertensive patients has been completed.

the laboratory animal, dietary restriction probably evokes disgust, depression, and sorrow, rather than anger, anxiety, and frustration But we do not know to what extent or in what direction the mood of animals is altered by these diets.

Technic of Sodium Depletion

While purging and sweating serve to deplete the sodium stores of the body, and can cause severe dehydration, these methods are no longer used, for they exhaust the patient and other less drastic methods are available. From the patients' point of view, the mercurial diuretics are the most convenient way to effect sodium depletion, and many patients can take large daily doses over long periods of time with no toxic effects and no renal lesions. Even when, on salt restriction, sodium excretion has fallen to very low levels, tubular reabsorption can be so impaired by mercurials that the daily sodium loss rises from less than 20 mg. to 5 Gm or more. Some patients do become sensitive to these agents; some fatalities have occurred at the time of injection. But it is safe to predict that, up to 1950, no more useful and dependable agent will be available for clinical use in promoting sodium loss from the body. The advantages of various preparations, dosage, and technic of use of mercurial diuretics in various disorders have been presented in detail recently (34) and need not be discussed here.

CATIONIC RESINS

Agents which have some promise are the cationic exchange resins, insoluble sulfonated synthetic resins which can exchange Na, K, Ca, NH_4, or H ions for any of these ions which are present in fluids to which the resin is exposed Thus a sodium resin, given by mouth, exchanges sodium for hydrogen in the acid gastric juice and hydrogen for sodium, calcium, and potassium in the gut. Washed with distilled water, the resin holds the H, Na, NH_4, or whatever ion it has picked up in the last exchange One resin, given by mouth, has taken up 4 per cent of its weight in sodium as it passed through the gastrointestinal tract of the rat In man, the best uptake per gram, of which we are aware, has been somewhat less Resins used in chemical laboratories and industries have sodium capacities of 10 per cent or more, and a steady increase in capacity of new products has been occurring as a result of research in this field.

When acid resins with an uptake of 1 per cent or less are taken by mouth, after being washed with distilled water, they are tasteless and cause no distress. Acid resins with a capacity of 6 per cent or more take base out of the fluid so fast that some burning is felt in the mouth and there may be real distress in the esophagus and the abdomen. For this reason high capacity resins would have to be used as potassium or ammonium compounds. A blend of acid, calcium, potassium, and ammonium resins might be used. This would not deplete the body store of potassium or calcium and would be free from unpleasant side effects

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The excretion of ammonium is increased when acid resins are given, and chloride is lost in the urine as base is taken out through the intestine. If resins were given as potassium salts, the acid or ammonia excretion would be less disturbed than when acid resins are given Each type of resin must be studied for its effect on the electrolyte metabolism It seems doubtful whether there will be much of a field for resins with a sodium exchange capacity under 5 per cent of their weight. A therapeutically available resin, without side effects, which could take up 10 per cent of its weight in sodium would revolutionize the sodium depletion problem Ordinary palatable diets could be taken, together with 20 to 40 Gm of resin per day. This is the goal of present research in the application of exchange resins in medicine. If achieved, it will permit study of sodium depletion uncomplicated by psychologic support or psychologic insult

DIET

Diet now plays a greater role than formerly in the program for salt depletion While it minimizes the need for mercurials, diet adds new embarrassments and often strains the will of the patient and leads him to seek other forms of control for his disease Only when some other feature is combined with sodium restriction can diet be justified in many cases in which sodium depletion can be effected by mercurials plus withholding the addition of salt to cooked or raw foods. Blood cholesterol cannot be lowered by any form of medication, the rice regime lowers blood cholesterol more effectively and dependably than any other reported regime (31, 11) Several regimes, low in salt, also lower calorie and protein intake, and this may be essential to the patients' welfare Once the patient must be on a special diet, some degree of sodium restriction is simple to effect, rigid restriction requires very special selection and handling of food

With the diet entirely free of sodium, the patient with normal renal function and normal sweat loss under basal conditions in a temperate climate, will have a negative sodium balance of 100 to 300 mg. per day At this rate, in 10 days he would lose the salt in 1 L of extracellular fluid This can be accomplished overnight with a small dose of mercurial diuretic With 400 mg of sodium (1 Gm of salt) in the daily regime, many hypertensive and anasarcous

patients remain in sodium balance, no depletion is effected by diet of this sort in such cases. On the other hand, in many cardiac patients, limitation of salt intake to 2 or 3 Gm has a striking effect on the patient's sense of well-being and his need for mercury. Such happy situations should not deceive doctors into thinking that salt depletion can be effected with any regularity unless the diet and medication provide less than 200 mg sodium per day

Obviously, when patients are put on low-salt regimes all sodium-containing medicines and proprietaries must be stopped, use of prepared foods made with salt, sodium benzoate, or baking powder must be discontinued, and salt and soda must not be used in cooking. "Salt-free" or salt-poor bread is now provided by some bakers, and some of the packaged breakfasts cereals are low in sodium, but all standard brands of bread, crackers, cakes, and candies contain added salt Most canned fruit juices (but not tomato juice!) are low in salt Most canned vegetables and meats are salty Most butter and oleomargarine is salted, but these can quickly be made salt-free by dropping in hot water, stirring after melting, allowing to solidify in the refrigerator, and then punching holes at the edges of the solid fat and pouring off the water The salt content of meat can be reduced if the ground meat is gradually added to boiling water, one quart to the half-pound of meat, and the water strained off after half an hour Root vegetables, relatively rich in salt, can be sliced and soaked in cold water over night, and leafy vegetables can be boiled in a large volume of water Such procedures reduce the vitamin and protein content of vegetables and meat, and make them relatively tasteless The taste can be restored to some extent by sautéing with a trace of butter or margarine, and adding very small amounts of salt substitutes now on the market—potassium, ammonium and calcium salts in various combinations Lithium and potassium, however, both may be toxic to patients on low sodium intake, so that spices are safer

While the rice diet enthusiasts bar all spices and the use of strongly flavored vegetables as flavoring or any of the sodium-free salt substitutes, most physicians who want patients to stay on low-sodium regimes indefinitely encourage trial of the different salt substitutes, and permit small amounts of spice, paprika, chili, onion, garlic, and other highly flavored natural products Coffee,

tea, and cocoa are permitted; chocolate usually is salted but may be desalted like butter; Dutch process cocoa is salted and can not be desalted. Most cola drinks are low in sodium. In some cities, the water has 5 to 35 mg. sodium per 100 cc., and distilled water must be used for drinking and cooking.

The physician or dietitian who consults the standard tables of foodstuffs finds variations of more than 100 per cent in the sodium figures for a given product in Sherman, in McCance and Widdowson, and in the Mead-Johnson list. The Mead-Johnson values are low—often less than 10 per cent the value in the old lists Rather than offer any absolute values, it seems wiser to point out main trends in salt content.

Unsalted nuts and peanuts provide fat- and protein-rich food with less than 5 mg. sodium per 100 calories Berries, apples, cherries, apricots, peaches, pears, grapes, and citrus fruit provide bulk, with low protein content and less than 10 mg. per 100 calories Rolled oats, and other milled grains, rice, shredded and puffed wheat, and puffed rice provide starch, some protein, and less than 10 mg. sodium per 100 calories Bread, cake, cookies, crackers, and processed and packaged cereals for cakes or breakfast food usually have 150 to 200 mg sodium per 100 calories and cannot be used at all in these diets Macaroni, spaghetti, arrowroot, and tapioca contain less than 5 mg. sodium per 100 calories and the last two are very low in protein—less than 1 Gm. per 100 calories, as compared to 8 in rice, 10 or more in the other cereals, and 14 in oats These items of diet, with protein content varying from over 25 Gm per 100 calories in peanuts to 0 5 Gm in tapioca, permit the planning of diets with a wide range of calorie and protein contents, great range in bulk and laxative effects, but always low in vitamins and flavor Diets based solely on this food list will contain from 100 to 200 mg sodium for a 2,000 to 2,500 calorie diet

Peas and beans, with a high protein and calorie content, range up to 25 mg sodium per 100 calories; root and leafy vegetables vary greatly, depending on the soil, but nearly all run over 20 mg per 100 calories, and some, like celery, are as high as 600 mg per 100 calories. Melons and tomatoes have over 100 mg per 100 calories Unless thoroughly leached by cold water, none of the vegetables just named should be used in a low-salt diet Meats contain 20 to 30 mg.

sodium per 100 calories; milk and eggs, 70 to 90 mg. per 100 calories, fish over 100 mg, shellfish over 500 mg After boiling, all forms of meat and egg can be leached free of salt, with little loss of protein. Sodium-free milk powders are now on the market, and are a great help in preparing many items of food In theory, then, nearly all the major food products can be used, after proper preparation, in a diet with less than 200 mg sodium per day, and even with a high calorie, high protein content. In practice, the desalted foods are unappetizing until patients, properly enthusiastic as to their value, learn to like them Fortunately, people can learn to like every kind of food, from the dry processed cereals Americans eat for breakfast to grasshoppers and the highly spiced foods of the Mexican and the rancid raw meat prized by the Eskimo They may even get used to taking an adequate salt-free diet. Usually this is more readily accomplished with a diet based largely on rice, fruit, and nuts than when desalting of a wide variety of foods adds to the cost and trouble of preparation. Rice also is said to have a protein better suited to mammalian needs than other grains, per gram of protein it supports growth of rats twice as well as wheat protein (36). Cottage cheese, made salt-free by acid precipitation and washing, also provides protein of high biologic value, while the proteins of beans and peanuts apparently are less desirable than those of grain

Since low-sodium diets may meet permanent therapeutic needs, the patient or a responsible member of the family must be instructed in the principles and in the practical details of preparing as palatable and adequate a diet as possible In general, such diets are far more difficult to manage and to maintain than are diabetic or reducing diets, and can only be compared with diets for people with food sensitivity This latter is not rare in hypertensive individuals, and may contribute either directly, or by causing some annoying symptom, to maintaining the high vasomotor tone. Citrus fruits may have to be omitted from the rice-fruit regime; fortunately rice, unlike wheat, milk, eggs, coffee, and chocolate, is rarely a factor in food allergy Some of the effectiveness of the rice-fruit regime, especially in heart failure and nephrosis, may be due to its simplicity and freedom from the usual food allergens On the other hand, for a housewife who enjoys cooking and eating, the virtue of a more complicated regime may lie in the psychotherapeutic value of feeling

that the day is taken up in useful labor and that, in preparing a variety of ingeniously thought-out and neatly executed dishes, there has been an opportunity to express her talent and skill. This may explain why Bryant and Blecha (15) had such excellent results with outpatients preparing their own 200 mg. sodium and 70 Gm. protein diets.

If the condition for which sodium depletion was initiated clears up completely, the diet is gradually made more liberal until evidence of recurrence appears Should none occur, or if symptoms and signs can be controlled by mercurial diuretics and the omission of table salt and salty foods, other dietary restrictions may be discontinued indefinitely. In an occasional patient, response to salt restriction may be prompt and complete in a few weeks yet with slow recurrence when normal diet is resumed. But even in these patients, a permanent change to low-sodium diets seems wise, while in cirrhosis, heart failure, or hypoproteinemia the objective of therapy is to free the patient of the need for restriction in his diet Even in these conditions it may be found necessary to maintain rigid dietary control to avoid frequent relapses

Results and Indications

In a very small percentage of a series of patients with hypertension put on low-salt regimes, the blood pressure falls rather quickly to normal, only to rise equally promptly if normal diet is resumed. In Schroeder's experience (10), most of these have been women presenting some of the features of Cushing's syndrome; in our series most of them have been Negro women, with no stigmata of Cushing's disease but with early onset of heart failure in hypertension None of these cases have been tested by determining electrolyte content of sweat to establish whether a desoxycorticosterone overactivity was present In a somewhat larger group, still not totaling 20 per cent of our cases, arterial pressure falls gradually to normal on long-continued salt restriction and has been shown to rise when salt alone has been added to the diet In many reported cases, salt loading has not been tested in cases in which pressure fell to normal; in others pressure has risen very slowly when normal diet was taken and did not return to the pretreatment level (33) In the majority of hypertensive patients staying indefinitely on the

rice regime, or a daily sodium intake of less than 200 mg., pressure remains above normal In one-third of the total, there is no real fall; in the others, pressure may fall strikingly or moderately.

The relative success of various regimes for sodium depletion suggests that with each 200 mg increment of sodium above the 200 mg. base line, the proportion of cases strikingly benefited is halved. Thus, if 60 per cent be taken as the proportion responding well to a 200 mg intake, 30 per cent might do well on 400 mg, less than 20 per cent on 600 mg, and less than 10 per cent on 800 mg per day. Since the patient may remain in sodium equilibrium, or store sodium, when the intake is over 200 mg, it is perhaps more surprising that some good results were noted, rather than that poor results were the rule, with most of the so-called low-salt diets used prior to 1940

Sodium depletion, used for any of the conditions mentioned in the second paragraph of this paper, seems to be purely a palliative in the great majority of cases in which it has any effect whatever. In the hypertensive patient, its effects may be compared with those of bed rest in advanced pulmonary tuberculosis It may reduce the pressure level, as rest reduces the fever; it may halt the progress of disability and of organic damage Maintenance of a low salt intake is not incompatible with an active life as is the bed rest needed to retard tuberculosis In this respect, sodium depletion is more effective in restoring normal health to the hypertensive than bed rest is for the tuberculous

Pheochromocytoma can now be detected by pharmacologic tests, and should be ruled out before diet is started Surgical intervention, psychoanalysis, and other traumatic, hazardous, or time-consuming forms of management should not be used until it has been clearly shown that a regime of sodium depletion, carried out for at least two months, has no real effect on the pressure level Where renal damage is present, the blood urea level must be carefully followed during sodium depletion, and salt given if urea begins to rise

As a rule it seems best to begin with a very simple low-protein diet, such as the rice regime, because it does reduce the chance of uremia developing, and patients prefer to have a diet made more liberal, when it proves effective, rather than have it become progressively more rigorous because it is ineffective The rigorous re-

gime also is an elimination diet, in the sense used by the allergist, and if it proves effective and is liberalized in progressive stages, the role of salt, of specific foods, and perhaps of diet as a psychotherapeutic agent can be evaluated for each case. On the other hand, if the rice–fruit regime combined with mercurial diuretics is given under conditions which evoke the maximum degree of hope and cooperation and fails to affect the level of blood pressure, the patient can be assured that his form of hypertension does not call for dietary restriction It may then be worth while considering sympathectomy or some other therapeutic approach if disability is present or if sight or life is threatened.

Dietary management of this sort, or sodium depletion by an agent which the patient could take with minimal expense and absence of side effects, is perhaps the only management which can be justified for the asymptomatic hypertensive patient with normal retina, kidneys, and heart. Many such patients remain free of symptoms for decades, with no therapy, or with reassurance and mild sedation Sodium depletion is the most effective therapy in cases with heart failure or with moderate degrees of kidney damage, primary or secondary. When sympathectomy fails, it is not possible to restore the patient to his condition prior to operation; but one can always go back to the normal diet Absence of the sympathetic chain is highly unphysiologic, reduction of salt intake to 100 mg. sodium per 1,000 calories is physiologic, for such has been the level of sodium ingestion of anthropoid apes and of billions of human beings.

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Clinical Use of Anticoagulants

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An adequate amount of circulating blood is necessary for the maintenance of health of man An intricate physiologic mechanism, involving several organs, is required to provide for the formation and circulation of blood An additional means, however, is necessary to prevent the loss of blood from the body when the continuity of blood vessels is interrupted This protective mechanism includes constriction and contraction of blood vessels, and the plugging of the rent in the wall of the vessel with platelets, these maneuvers, however, are of relatively minor scope in most instances The major action in preventing loss of blood from the body is the conversion of the physical state of the blood from a sol to a gel at the openings in the vascular network. Without this mechanism, human life of any significant duration would be impossible, yet, the mechanism contains a basic defect which is responsible repeatedly for the deaths of individuals whom it was designed to protect This defect is the change of blood from the sol state to the gel state *within* the vascular network; namely, intravascular coagulation or thrombosis

Intravascular thrombosis results in occlusion of the affected blood vessel, and thereby interferes with the circulation of the blood The clinical syndrome resulting therefrom depends on the site of the occlusion (arterial or venous), the organ involved, and the unaffected collateral vascular channels of the organ Should this defect result in failure of function of a vital organ, death occurs Even if death does not supervene as a result of intravascular coagulation of blood, there remains a defective blood flow, since collateral circulation is seldom entirely adequate In addition, there is danger of embolism—a breaking away of a blood clot and its transportation to another portion of the body This may result in death or in a residual defect of blood flow in the new location Thus, although man is provided with a

abnormalities which favor intravascular coagulation They are used in clinical practice for two general purposes: to prevent intravascular embolism or thrombosis or to prevent extension of thrombosis

Because there is no adequate method for predicting the occurrence or progression of intravascular thrombosis or embolism, many persons receive anticoagulants who do not actually need them. The physician who uses anticoagulants must treat many patients to save the lives of a few and to spare a few more patients the consequences of progressive thrombosis. This situation will persist until some method is developed by means of which the occurrence of intravascular thrombosis can be predicted, as, for example, following a major surgical procedure; or until it is possible for the physician to predict with accuracy whether or not a minor episode of thrombosis, for example, in the sural veins, will progress or cause pulmonary embolism Until these goals are achieved, the physician will need to treat prophylactically those patients who, as indicated by clinical experience, are likely to have intravascular thrombosis and those who already have intravascular thrombosis The only alternatives are to ignore the advantages of treatment with anticoagulants, to use some other method of treatment such as ligation of veins, or to accept the consequences of intravascular thrombosis A major challenge to the medical profession today is the development of a means of determining the *likelihood* of intravascular thrombosis before the latter actually occurs.

Heparin

Heparin is a substance with anticoagulant properties which was described by McLean in 1916, and named by Howell and Holt in 1918 to indicate its hepatic origin The chemical nature of heparin is complicated, and a discussion of it is not within the scope of this presentation (3,10–18,20,21). Heparin may be found in large quantities in the liver, lungs, and muscle. To a lesser extent, it is present in the spleen, heart, and thymus Negligible amounts are found in serum According to Quick (34), the anticoagulant action of heparin is threefold. (*1*) prevention of the conversion of prothrombin to thrombin in the presence of a plasma cofactor, (*2*) action with serum albumin to form an antithrombin, (*3*) prevention of liberation of thromboplastin from blood platelets.

mechanism which induces coagulation of blood and prevents its loss, this same mechanism of coagulation makes possible abnormal intravascular thrombosis of the blood, which is deleterious. In the following pages certain syndromes resulting from thrombosis or embolism will be presented. The therapeutic and prophylactic use of anticoagulant substances in these states will be discussed.

Rationale of Anticoagulant Therapy

The occurrence of intravascular thrombosis, except that which prevents bleeding, is distinctly abnormal. It may infrequently result from trauma or from an inflammatory or degenerative process (angiitis or arteriosclerosis). It appears in some instances to be related, at least in part, to diminished speed of flow of blood in veins, as for example, that which occurs postoperatively (35). In other instances intravascular thrombosis seems to represent a disturbance of the intricate factors which maintain fluidity of blood, although there is as yet no wholly acceptable proof that increased coagulability exists. This is probably due to the inadequacy of methods for studying coagulation of blood. Certainly it is folly to believe it possible to measure precisely the coagulability of blood inside blood vessels by studying coagulability in an abnormal environment such as a glass tube Determination of coagulability of the blood outside the body is in some degree a measure of the abnormality of the environment rather than a measure of coagulability. Blood coagulates more rapidly in glass tubes than it does in plastic tubes and more rapidly in plastic tubes than it does in tubes coated with silicone. Moreover, it seems highly probable that blood cannot be drawn from the body without interfering with the mechanism normally operative inside blood vessels

In many instances the cause for intravascular thrombosis cannot be determined Whether or not blood will coagulate in a blood vessel seems to represent the dominance of factors which encourage coagulation of blood or the dominance of factors which favor the persistence of the fluid state For example, arteriosclerosis does not always cause intraarterial thrombosis, it does so only when the local stimulus for coagulation overcomes the natural resistance of blood to intravascular coagulation. Anticoagulants are used for the specific purpose of maintaining the blood in a fluid state, regardless of the

abnormalities which favor intravascular coagulation They are used in clinical practice for two general purposes: to prevent intravascular embolism or thrombosis or to prevent extension of thrombosis.

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When 50 mg of heparin are injected intravenously, a maximal anticoagulant effect occurs within a few minutes For about 1 hour the coagulation time remains at about 4 times normal; it then decreases to normal in about 3 hours The anticoagulant effect of heparin may be counteracted by a protamine; namely, salmine Parkin and Kvale (32) have found that 1 5 mg of salmine will neutralize 1 mg. of heparin *in vitro*. *In vivo* tests show that this ratio changes so that 1 mg. of salmine will neutralize 1 mg of heparin (Fig 1).

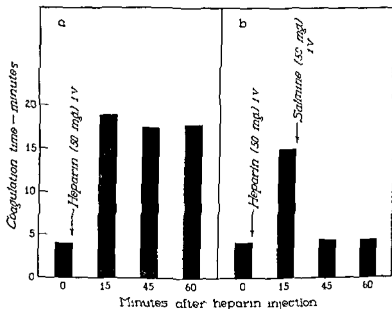


Fig. 1. The neutralization of the anticoagulant effect of heparin by salmine (32).

Clinically, heparin may be given in 3 ways· (*1*) intermittent intravenous injection, (*2*) continuous intravenous injection; (*3*) intramuscular or subcutaneous injection, either in its normal state or incorporated in a menstruum. The continuous intravenous infusion of heparin, although theoretically ideal from the standpoint of continuous anticoagulant effect, poses certain problems which make this mode of administration inconvenient and at times impractical. Usually 200 mg of heparin are added to 1,000 cc of diluent, preferably a 5 per cent solution of dextrose, and administration is

begun at the rate of 25 drops per minute. The number of drops per minute is varied as necessary to maintain the coagulation time of venous blood, between 15 and 25 minutes, as determined by the Lee-White method This requires that the blood coagulation time be determined every 6 hours for the first 24 hours and every 12 to 24 hours thereafter, since tolerance to heparin may vary considerably among different persons and even from day to day in the same patient. We have used continuous heparinization for longer than 2 weeks, but we have found it an unwieldy method which does not seem more advantageous than intermittent intravenous injection.

In the intermittent intravenous administration of heparin, the patient receives intravenous injections of a concentrated solution of heparin. We usually inject 50 mg every 4 hours Some clinicians omit the injection which might ordinarily be given between 10 P.M. and 6 A M. and give, for example, 100 mg at 10 P.M. and no more until 6 A M. Other clinicians give as much as 150 mg. at a single injection and as infrequently as twice daily We have had no experience with this latter program of treatment Treatment by the intermittent injection of heparin ignores differences in tolerance to heparin and also the fact that there is little or no demonstrable effect on the blood coagulation time for periods longer than 2 to 3 hours after injection However, in clinical practice this method seems to be as effective as continuous intravenous injection, which we have abandoned almost entirely When heparin is given intravenously and in undiluted form (50 mg in 5 cc) it is not necessary to determine the blood coagulation time during the course of treatment

Our experience with the intramuscular injection of heparin in a menstruum has not been satisfactory, chiefly because of pain at the site of injection Recently, Loewe and Eiber (25) reported that a newer preparation has overcome this objection, and that other side effects of the heparin-Pitkin menstruum are trivial They recommended the subcutaneous injection of 400 mg of heparin in a menstruum at intervals, in general, of every 2 days These authors noted prolongation of coagulation time within 1 to 2 hours of injection, and persistence of it for 48 hours or longer Absorption of heparin was slowed by the application of an ice bag to the area of injection or application of a tourniquet above Small transfusions of whole

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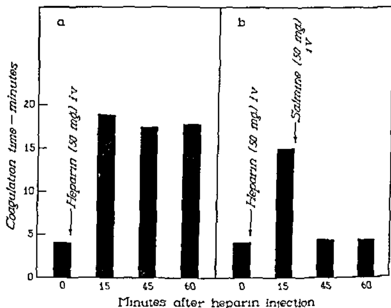


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dicumarol or for avoiding it, because the effect of dicumarol is enhanced and prolonged by such failure; it is not, however, a contraindication to the use of heparin

Dicumarol

The splendid researches of Link (24) and his associates in the isolation and synthesis of dicumarol are too well known to require detailed narration here. Their contributions constitute one of the truly great chapters in the annals of man's fight against disease. Dicumarol was first administered to man by Bingham, Meyer, and Pohle (8), and somewhat later by Butt, Allen, and Bollman (9), who did not know of the former's work. The first clinical report published was that of Butt, Allen, and Bollman

Dicumarol is 3,3'-methylenebis(4-hydroxycoumarin). It is a white crystalline powder slightly soluble in water, but readily soluble in alkaline solutions

Currently, the anticoagulant effect of dicumarol appears to occur as a result of a decrease in the activity of prothrombin in the blood plasma This is manifested by an increase in the prothrombin time. We use the Magath modification of the Quick prothrombin time test, but other clinicians use other tests for prothrombin. According to the technic used in our laboratories, 18 to 21 seconds represents normal prothrombin activity; 27 seconds, 30 per cent prothrombin activity; 35 seconds, 20 per cent prothrombin activity; and 60 seconds, 10 per cent prothrombin activity. In the discussion which follows we shall designate the values as percentage of normal prothrombin, although it is improbable that they represent quantitative determinations.

The method for determining prothrombin time is unimportant, provided the prothrombin time reported by the laboratory can be converted into percentage of prothrombin in the blood Since thromboplastins vary in potency, some laboratories which do not perform many tests of prothrombin time may have difficulty in obtaining a consistent value when normal plasma is studied Some clinicians consider it safe to maintain prothrombin time with dicumarol within a range of 2 to 2 5 times the value for normal plasma, provided the value for normal plasma does not exceed 20 seconds. The values for prothrombin activity with this method would be about 15 to 20 per cent with the method used in our laboratory.

blood or relatively fresh bank blood inactivated any circulating heparin. Loewe and Eiber considered this method of using heparin to be very satisfactory even over long periods.

The effectiveness of heparin in the prevention and treatment of thrombosis and embolism has been demonstrated in large series of patients. Murray (28) has reported treatment of 440 postoperative patients with heparin without the occurrence of a pulmonary embolism; 29 patients who survived an initial pulmonary embolism were treated with heparin without subsequent fatality. Bauer (7) has reported treatment with heparin of 209 postoperative and nonoperative patients who had "acute deep leg thrombosis." In this series, there were no postoperative deaths from pulmonary embolism; 3 nonoperative patients had fatal pulmonary embolisms. Bauer considered the mortality rate from thrombo-embolism to be reduced to one-tenth of that when heparin is not used. Jorpes (22) has summarized the use of heparin by several clinicians in several hundred patients. He states that there has been a marked decrease in mortality rate and morbidity from thrombo-embolic phenomena in patients who have received heparin as compared with patients who have not received it

If heparin is administered, the possibility of abnormal bleeding exists, especially in operative incisions when administration is begun in the first 12 to 24 hours after operation. Actually, hemorrhage due to administration of heparin is rare. If immediate neutralization of the effect of heparin is deemed necessary, because of hemorrhage, salmine may be given intravenously in a dosage of 50 mg. (Fig. 1). When heparin has been administered in a menstruum, the effect of salmine may disappear before that of the heparin Therefore, when bleeding is occurring as a result of administration of heparin in a menstruum, blood coagulation times should be obtained and additional salmine administered, if required, to maintain a normal coagulation time.

The indications and contraindications for the use of heparin are presumably the same as for the use of dicumarol, which will be discussed subsequently However, we have used dicumarol so extensively that we have learned much from clinical experience We have used heparin much less extensively and are less certain of the contraindications to its use. Renal failure is a reason for care in using

bleeding tendency, because of increased tendency to bleed; (4) lack of trained laboratory personnel and of adequate facilities for determining values for prothrombin Caution should be employed in the use of dicumarol or the drug should be withheld when there are ulcerating lesions of viscera or skin because of danger of hemorrhage. There is some danger from hemorrhage when dicumarol is given to patients who are being treated with Wangensteen or Miller-Abbott tubes, or to patients with diffuse hepatic disease. Renal failure with azotemia is an indication for extreme care in the use of dicumarol because renal failure enhances and prolongs the effect of dicumarol In a considerable number of instances, as, for example, that of a man with acute myocardial infarction and mild symptoms of duodenal ulcer, the physician will need to decide about the administration of dicumarol on the basis of probable harm and probable good, the decision may be difficult.

In most instances, dicumarol is ingested in an initial dose of 300 mg. In other cases 200 mg. may be administered initially. There is no absolute rule to follow. Prothrombin time is determined the following day, and unless the concentration of plasma prothrombin has shown a marked decrease, another 100 or 200 mg. are given on this day. The effect of dicumarol usually becomes manifest on the second to fourth day after administration After it becomes effective, the dosage of dicumarol depends on the concentration of plasma prothrombin Our clinical experience indicates that the desirable therapeutic range varies between 10 per cent and 30 per cent of normal prothrombin concentration Other clinicians believe that less marked reduction in prothrombin is as effective in preventing intravascular thrombosis There can be no definitive answer relative to the most desirable concentration of prothrombin The occurrence, or failure of occurrence, of intravascular thrombosis is dependent on two factors: the stimulus to intravascular coagulation on the one hand, and the resistance to loss of the fluid state of the blood on the other Obviously, if the stimulus to intravascular thrombosis is mild, a minor reduction in prothrombin concentration might prevent it, whereas a minor reduction of prothrombin in the blood might permit intravascular coagulation if the stimulus were great.

We adhere to the program which follows, simply because it has worked well in our experience. On days when the prothrombin con-

We wish to emphasize that use of dicumarol is entirely inadvisable unless the laboratory is equipped with equipment and personnel to render wholly reliable reports. This constitutes a *sine qua non* of treatment with dicumarol If such reports cannot be obtained, it is best to use heparin when treatment with an anticoagulant is desirable. To administer dicumarol without adequate and reliable laboratory control is to court disaster from hemorrhage, or to treat a patient inadequately

There exists a distinct variability in the decrease in prothrombin concentration in the blood plasma of different individuals receiving identical doses of dicumarol, and the same amount of dicumarol given to the same patient on different occasions may affect prothrombin times dissimilarly The explanation for this situation is obscure. It may be due to differences in absorption of dicumarol, to varying effect on prothrombin of identical amounts of absorbed dicumarol, to varying amounts of vitamin K present in different individuals, to variable rates of excretion of dicumarol, or to a combination of these factors, all of which are theoretical The variability of response of prothrombin to identical amounts of dicumarol is one of the disadvantages of treatment with it It makes treatment with dicumarol entirely dependent on adequate laboratory studies

The effect of dicumarol may be nullified by the administration of menadione bisulfite (synthetic vitamin K) in doses of 36 to 72 mg The amount of menadione bisulfite necessary to return the plasma prothrombin to normal cannot be exactly computed In the event of abnormal bleeding or a concentration of plasma prothrombin well below 10 per cent, it is well to administer 72 mg In the presence of hemorrhage, this dose may be repeated at 4-hour intervals until the plasma prothrombin is of normal concentration To correct abnormal plasma prothrombin concentrations of less severity, 36 mg. may be administered Transfusions of blood, preferably fresh, will also restore the prothrombin time to normal when it has been prolonged by dicumarol

Contraindications to the use of dicumarol may be summarized as follows: (*1*) subacute bacterial endocarditis, because of the danger of cerebral hemorrhage; (*2*) recent operation on the brain or spinal cord, because of the severe damage which might follow hemorrhage in these regions, (*3*) purpura and blood dyscrasias with

effect on the blood. Its effect on blood coagulation *in vivo* is short-lived—about 2 to 3 hours as measured by the Lee-White test; theoretically, therefore, prolonged bleeding is not to be expected. It may be administered intermittently by intravenous injection, laboratory control is not necessary, and its effect may be immediately neutralized by an injection of salmine However, the fact that heparin must be administered parenterally is a disadvantage. Also, it is expensive, the cost of administration at present being about $9.00 to $15 00 per day. For these two reasons, prolonged treatment with heparin

TABLE I

Incidence of Bleeding among Patients Treated Postoperatively with Dicumarol (1)

Reasons for dicumarol therapy	Total patients treated	Minor bleeding		Major bleeding	
		Number	Per cent of total	Number	Per cent of total
Postoperative pulmonary embolism	180	5	2.8	2	1 1
Postoperative thrombophlebitis	138	5	3 6	1	0 7
History of thrombosis or embolism at any time prior to operation (prophylaxis)	61	1	1 6	1	1 6
Abdominal hysterectomy (prophylaxis)	438	20	4 6	12	2.7
Other operations (prophylaxis)	183	8	4 4	9	4.9
Total	1,000	39	3 9	25	2 5

is disadvantageous. Dicumarol, on the other hand, may be administered *daily* by mouth It costs only $0 045 for 100 mg ; the cost of a prothrombin time determination is $3 00 to $5 00 in most laboratories; dicumarol is therefore considerably cheaper to administer than heparin The effect of dicumarol, once established, may last for days; this is an advantage, since it may be given much less frequently than heparin, which is administered intravenously. On the other hand, this prolonged effect is disadvantageous because, in case of bleeding, the prothrombin time must be restored to normal by means of menadione bisulfite or by transfusion, whereas the coagula-

centration is greater than 20 per cent, 100 or 200 mg. of dicumarol are given. The choice between doses of 100 and 200 mg depends on the sensitivity of individual patients to the drug On a day when the prothrombin concentration is less than 10 per cent, menadione bisulfite (synthetic vitamin K) may be given, if there is presumed to be danger from hemorrhage. Certainly the persistence of prothrombin values of less than 10 per cent for two days is an indication of need for the use of vitamin K.

It has been our experience that bleeding ordinarily does not occur when the prothrombin concentration is greater than 10 per cent. However, it is well to know that there is *no certain way to avoid hemorrhage if dicumarol is used.* We have observed that hemorrhage may not occur in the presence of great prothrombin deficiency and that it may occur when there is relatively little prothrombin deficiency. This is understandable, since factors other than prothrombin value are responsible for bleeding or for the absence of it Also, we have noted that thrombo-embolic episodes rarely occur when the concentration of prothrombin is less than 30 per cent There is, however, no absolute assurance that intravascular thrombosis or embolism will not occur when prothrombin values are less than 30 per cent. If the stimulus to coagulation is great, it may occur inside blood vessels even when prothrombin is sharply reduced in amount We can state only that, judging by clinical experience, it seems best to maintain the prothrombin at values less than 30 per cent and greater than 10 per cent Table I summarizes the incidence of bleeding among postoperative patients given dicumarol.

Should bleeding occur, menadione bisulfite should be given intravenously in the manner already indicated. Should the bleeding be of major type, transfusion with blood replaces blood lost by hemorrhage. Recently, it has been shown that vitamin K_1 oxide apparently corrects the prothrombin deficiency over a period of about 6 hours, rather than over the 12 to 24 hours required for menadione bisulfite to correct it Further work is required with this substance before final opinions as to its efficacy can be formulated

Comparison of Heparin and Dicumarol

Each substance has its own advantages and disadvantages Heparin has the advantage of producing an immediate anticoagulant

patients treated with dicumarol reveals that minor bleeding occurred twice (0 7 per cent) and major bleeding 3 times (1.0 per cent). In the latter group were 2 instances of gastrointestinal bleeding and 1 instance of severe subcutaneous bleeding. There were no deaths.

Heparin and Dicumarol Not Ideal Anticoagulants

Extensive experience at the Mayo Clinic has indicated that heparin and dicumarol are useful in clinical practice However, the use of either alone or the two together, particularly over long periods, is far from satisfactory. Heparin needs to be given parenterally and requires injections by medical personnel; it is expensive. Although dicumarol may be administered by mouth, treatment with it calls for repeated determination of prothrombin values in the blood; ideally, the determination should be accomplished daily, although in some instances after prolonged observation and treatment weekly determinations may be satisfactory. It is difficult or impossible to establish a *maintenance dose* of dicumarol, for identical amounts of dicumarol affect the prothrombin of the same and of different individuals to a variable degree It seems to us that treatment with anticoagulants will not be wholly satisfactory until one is available which is cheap, which can be administered orally, and which, in identical amounts, will always have identical effects on prothrombin or coagulation Until this desideratum has been achieved, clinicians will need to be satisfied with less than an ideal situation

Indications for Use of Anticoagulants

In general, the use of anticoagulants is indicated when undesirable intravasculuar thrombosis or the probabilities of it exist We have used anticoagulants in the following conditions (*1*) thrombophlebitis and venous thrombosis in the extremities, (*2*) pulmonary embolism, (*3*) myocardial infarction; (*4*) acute and chronic peripheral arterial occlusion, (*5*) cerebral thrombosis; (*6*) thrombosis of a retinal vein We have also used anticoagulants in anticipation of vascular thrombosis or embolism in conditions which predispose to vascular thrombosis or embolism, as follows (*7*) postoperative, (*8*) postpartum, (*9*) congestive heart failure, (*10*) auricular fibrillation, (*11*) vascular surgery

tion time which is prolonged by heparin injected intravenously quickly returns to normal. Dicumarol has the further disadvantage that its effect on prothrombin time appears only 24 to 72 hours after administration. In many cases of embolism or thrombosis the clinician cannot wait so long, as intravascular thrombosis must be halted without delay. Lastly, with dicumarol, laboratory facilities and personnel for adequate control of dosage are essential.

Concurrent Use of Heparin and Dicumarol

The concurrent use of heparin and dicumarol may be desirable. Heparin is injected intravenously in doses of 50 mg. every 4 hours. Administration of dicumarol is begun on the same day. The value for prothrombin is determined daily; when the anticoagulant effect of dicumarol becomes manifest, no more heparin is injected. As heparin injected into the blood prolongs prothrombin time (26), venipuncture to secure blood for study of prothrombin content should not be performed before a lapse of at least 3 hours after an injection of heparin.

Danger of Hemorrhage

Whenever heparin or dicumarol is used in therapeutic doses there is some danger of hemorrhage. We have not used heparin over a sufficiently long period in a sufficiently large number of patients to know the incidence of bleeding following its use, but Loewe and Eiber (25) did not report any instances of hemorrhage when there is an "intact cardiovascular system." We have observed bleeding during use of heparin only when it has been administered shortly after operation. In a series of 1,983 postoperative patients given dicumarol, our associates and we have noted that minor bleeding (epistaxis, hematuria, or localized ecchymosis) occurred in 3.4 per cent of cases, and that major bleeding occurred in 1.8 per cent of cases. There is, of course, a great difference between major bleeding and fatal bleeding. Although marked bleeding occurred about 40 times during the course of treatment of almost 2,000 patients who had undergone operation, death from hemorrhage occurred but twice. Careful study of these 2 fatalities indicates that the fatal hemorrhage could not definitely be attributed to the effect of dicumarol. A study of the incidence of bleeding in 288 "medical"

patients treated with dicumarol reveals that minor bleeding occurred twice (0 7 per cent) and major bleeding 3 times (1 0 per cent). In the latter group were 2 instances of gastrointestinal bleeding and 1 instance of severe subcutaneous bleeding There were no deaths.

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THROMBOPHLEBITIS

We are aware that venous thrombosis may occur as a result of phlebitis, as, for example, that affecting the superficial veins in thromboangiitis obliterans. It may also occur without phlebitis, as, for example, that occurring in the sural veins after a major operation. There has been a good deal of discussion about the terms "thrombophlebitis" and "phlebothrombosis," but both are anatomic diagnoses and the clinician may find it impossible to decide which exists. Nor is this necessary in most instances. Rationally, it is fair *to conclude that in thrombophlebitis the possibility of pulmonary* embolism is remote because the clot is anchored to the wall of the vein. Conversely, in phlebothrombosis it is rational to believe that pulmonary embolism is likely because the clot is not anchored to the wall of the vein. Actually it is unsafe and unwise to decide about the need for anticoagulants on the basis of such an uncertain hypothesis. With few exceptions, venous thrombosis is an indication for the use of anticoagulants regardless of the presence or absence of phlebitis. Indeed we are so little impressed by the practical value of attempting to differentiate thrombophlebitis from phlebothrombosis that we use the designation "thrombophlebitis" for both.

Well-developed acute iliofemoral thrombophlebitis is characterized by development of edema of the entire leg within a few hours. The skin usually has a cyanotic color, but it may be pale. Acute iliofemoral thrombophlebitis may occur after major operations, after childbirth; as a result of trauma and malignant neoplasms, including lymphomas, in congestive heart failure; in the course of prolonged rest in bed (especially of the aged); in severe infections; or in polycythemia vera Pelvic, gastric, pulmonary, or retroperitoneal malignant tumors are neoplasms which seem particularly prone to be associated with thrombophlebitis. At times, thrombophlebitis occurs without apparent cause

There are two considerations in the treatment of acute iliofemoral thrombophlebitis: the prevention of a pulmonary embolism, and the prevention of further thrombosis The latter may be in the form of extension of the clot at the original site or the formation of a new clot in another location

We advise the combined use of heparin and dicumarol for acute iliofemoral thrombophlebitis This program affords the patient the

best opportunity to escape pulmonary embolism and further venous thrombosis which increases the severity of subsequent chronic venous insufficiency.

The recognition of thrombophlebitis involving the veins of the calf may be difficult because of the absence or mildness of symptoms. The patient may complain of tenderness in the involved calf on pressure, or on dorsiflexion of the foot This tenderness may be noted when the postoperative patient first becomes ambulatory. Edema is absent or minimal. Examination may reveal tenderness of the calf when digital pressure is applied. Not infrequently, a tender, indurated area may be felt in the involved calf. This, no doubt, represents the area of thrombophlebitis The same considerations obtain in this instance as in iliofemoral thrombophlebitis Usually, however, we are content to use dicumarol, although theoretically it would seem best to use heparin and dicumarol concurrently until an adequate prothrombin deficiency is attained If there is evidence of progressive thrombosis in the sural veins after dicumarol has been administered and before there is reduction in the prothrombin, heparin should be used. The physician who sees many patients with venous thrombosis will encounter many who complain of tenderness in the sural region under circumstances which make venous thrombosis probable Frequently, we are unable to deny or prove the presence of venous thrombosis, in such instances we administer dicumarol alone as an anticoagulant, and this procedure has proved to be efficacious

The occurrence of thrombophlebitis in varices is fairly common, but the incidence of pulmonary embolism is small because of tortuosity of the varices and variations in the caliber of the lumen of the vein The two factors which influence the decision to use anticoagulants for thrombophlebitis in a varix are the nearness of the process to the greater saphenofemoral junction or to the lesser saphenopopliteal junction, and the possibility that the varicose thrombophlebitis may be associated with thrombosis in the deeply situated veins. If the thrombophlebitis is near one of the junctions of the superficial and deep venous systems, extension may occur from the superficial into the deep system and cause pulmonary embolism or cause chronic venous insufficiency after the acute phase has passed. It is well in this type of case to use the combined heparin-dicumarol

regimen in order to achieve immediate anticoagulant effect, or to resort to ligation of the varix. The possibility that thrombophlebitis in a varix may be associated with a tendency to intravascular thrombosis elsewhere constitutes the rationale for the use of anticoagulants even when there is no evidence of direct extension into the deep venous circulation. In thrombophlebitis in a varix we use dicumarol alone, provided there is no apparent danger of extension into the deep veins.

The occurrence of superficial thrombophlebitis in veins of normal caliber may occur in thromboangiitis obliterans, in a syndrome of unknown etiology called recurrent idiopathic thrombophlebitis, or in malignancy. Ordinarily we use dicumarol alone in this condition However, should an indication for rapid anticoagulant effect exist, heparin may also be used.

Axillary–subclavian thrombophlebitis may occur as a result of trauma, of pressure by a cervical rib, of pressure by muscles when the vein traverses an abnormal course, or of pressure by lymph nodes When it is due to the last named, the nodes are usually neoplastic The clinical syndrome of a swollen upper extremity, prominent superficial veins, and, frequently, palpable, tender axillary vein, is easily recognized Pulmonary embolism occurs very rarely with this condition Development of chronic venous insufficiency in the arm is also rare Ordinarily, we use dicumarol to prevent progression of the thrombus

Thrombophlebitis may occur in a vein used for intravenous injection of medications, such as that used in obtaining an excretory urogram. The resultant chemical thrombophlebitis usually has a marked inflammatory reaction; pulmonary embolism has occurred in 1 of our cases Unless there is evidence of pulmonary embolism, active progressive thrombosis or of thrombosis in the deep brachial–axillary–subclavian venous system we do not use anticoagulants in such cases.

The results of anticoagulant therapy in postoperative venous thrombosis are indicated in Tables II and III. It is apparent that the use of dicumarol does not afford complete protection However, the markedly lowered incidence of thrombo-embolic episodes speaks adequately for the efficacy of dicumarol The failure of dicumarol may be traced in some cases to inadequate lowering of the concentra-

TABLE II

Postoperative Thrombophlebitis: Anticoagulants Not Administered (1)

	Number	Per cent
Total cases	897	100
Subsequent episode of thrombophlebitis	95	10 6
Subsequent fatal pulmonary embolism	51	5 7

TABLE III

Results of Use of Anticoagulants in 352 Cases of Postoperative Venous *Thrombosis*

	Number expected if anticoagulants had not been used*	Number occurred
Subsequent venous thrombosis or pulmonary embolism	88	9†
Fatal pulmonary embolism	20	0

* In this table and in Table V the expected cases are calculated on the basis of the rates given in the reports of Barker, Nygaard, Walters and Priestley (5,6).

† In 3 cases, the prothrombin in the blood was more than 30 per cent. In 1 case, use of dicumarol had been discontinued and prothrombin was normal

tion of plasma prothrombin This is not true in other cases, in which the prothrombin activity was within a 10 to 30 per cent range In these instances it seems that the stimulus to intravascular thrombosis was great enough to overcome the effect of diminished prothrombin

PULMONARY EMBOLISM

Pulmonary embolism is a vascular accident of serious import, for it may cause sudden death, or it may herald further thrombo-embolic episodes which may cause death At the very least, pulmonary embolism prolongs convalescence from the postoperative or postpartum states or from medical diseases. Table IV shows the statistical evidence gathered by Allen, Barker and Hines (1) in a study of 678 cases in which nonfatal postoperative pulmonary embolism and infarction were diagnosed clinically The information summarized in this table confirms our previous statement that a

pulmonary embolism frequently heralds further thrombo-embolic episodes.

The insidiousness of the venous thrombosis which precedes a pulmonary embolism is well shown in the study of 343 cases of postoperative fatal pulmonary embolism by Allen *et al.* (1) In approximately 40 per cent of the cases of this series there was no clinical or postmortem evidence of venous thrombosis or thrombophlebitis, and in these cases it is likely that the entire thrombus became detached to form an embolus In approximately 45 per cent of the cases there was no clinical evidence of venous thrombosis or thrombophlebitis, but venous thrombosis was found at necropsy; in these cases the thrombus was fresh, and showed little evidence of organization. In only 15 per cent of the

TABLE IV

Nonfatal Postoperative Pulmonary Embolism and Infarction: Anticoagulants Not Administered (1)

	Number	Per cent
Total cases	678	100
Subsequent venous thrombosis, pulmonary embolism, or infarction	297	43 8
Subsequent fatal pulmonary embolism	124	18 3

343 cases was there clinical evidence of thrombophlebitis in any vein before death from pulmonary embolism. In only 5.2 per cent, or 18 cases, did iliofemoral thrombophlebitis antedate pulmonary embolism In 7 of these 18 cases the embolism came as a result of a detached thrombus in the opposite iliofemoral vein, which had not produced any clinical signs In the study of postoperative thrombophlebitis it was found that fatal pulmonary embolism occurred in only 6 6 per cent of the cases of iliofemoral thrombophlebitis, in 6 0 per cent of the cases of short saphenous thrombophlebitis, and in 0 6 per cent of the cases of long saphenous thrombophlebitis

The foregoing summary clearly shows that pulmonary embolism occurs relatively infrequently from clinically recognizable thrombophlebitis, but that it most frequently originates from insidious, symptomless, fresh venous thrombi Therefore, anticoagulant therapy of thrombophlebitis has as its major aim the prevention of fresh

venous thrombosis, either as an extension of the clot already present or as thrombosis at a different site. This form of treatment not only lowers the incidence of pulmonary embolism, but tends to decrease the severity of subsequent chronic venous insufficiency.

The clinical picture of a major pulmonary embolism is usually easily distinguishable This type usually produces marked signs and symptoms, including severe thoracic pain, dyspnea, cyanosis, shock, and a sensation of impending death. It may be suddenly fatal, the patient may live a few hours, or he may recover This major type of pulmonary embolism is, fortunately, more rare than the lesser forms.

The importance of recognizing the lesser embolic episodes lies in the fact that they may be followed by fatal embolism Unless the physician is on the alert for the possibility of minor pulmonary embolism it may be overlooked. Patients may complain only of vague thoracic distress, mild dyspnea, or minor pleurisy. Usually, in these lesser cases there is no hemoptysis, cyanosis, or shock, and dyspnea is minor or absent. The results of electrocardiographic and roentgenographic studies are frequently within normal limits A roentgenogram of the thorax may show a small area of increased density, but frequently this does not appear until after one or two days. A moderate increase in the pulse rate is noted sometimes. A localized area of moist crepitant rales, or a pleural friction rub, or both may be heard.

All forms of vague thoracic distress in postoperative, postpartum, or medically ill patients should be considered as possibly due to pulmonary embolism, and any objective clinical evidence suggestive of pulmonary embolism, no matter how slight, must not be ignored. Because of a high index of suspicion, some patients who have not actually had a pulmonary embolism will be considered to have had one and will be treated with anticoagulants This is preferable to ignoring minor signs and symptoms suggesting pulmonary embolism The risk of giving anticoagulants to a patient who has no contraindication for such therapy is minor, but failure to institute treatment is to risk the possibilities of fatal embolism

We administer both heparin and dicumarol to patients who have had a major pulmonary embolism In cases of minor pulmonary embolisms we usually are content to use dicumarol alone. When-

ever doubt exists as to the preferred course to follow, it is best to resort to the combined form of therapy. The results of treatment with anticoagulants in 329 cases of pulmonary embolism, reported by Allen *et al.* (2), are summarized in Table V The efficacy of anti-

TABLE V

Results of Anticoagulant Therapy in 329 Cases of Pulmonary Embolism

	Number expected if anticoagulants had not been used	Number occurred
Subsequent venous thrombosis or pulmonary embolism	144	3
Fatal pulmonary embolism	60	1*

* Occurred after prothrombin time had returned to normal.

coagulant therapy is clear, and with this form of treatment untimely deaths can be prevented. However, the ideal in the consideration of pulmonary embolism is the prevention of it This requires the prophylactic use of anticoagulants in conditions with a known predisposition for the occurrence of a pulmonary embolism or thrombophlebitis

PROPHYLACTIC USE OF ANTICOAGULANTS

The postoperative state has been the one most extensively treated with anticoagulants to prevent pulmonary embolism The experience at the Mayo Clinic has been that the incidence of clinically diagnosable thromboembolic disease is approximately 1 per cent following all operations and about 3 to 4 per cent following laparotomy with extensive resections The incidence of fatal pulmonary embolism is approximately 0 2 per cent following all laparotomies and 0 7 per cent in laparotomies with extensive resections

Factors which increase the incidence of occurrence of pulmonary embolisms in postoperative patients are old age, obesity, infection, malignant lesions, heart disease, varicose veins, and anemia or polycythemia A history of thrombophlebitis or pulmonary embolism at any time prior to the operation appears markedly to increase the usual risk of thrombo-embolism, although exact figures are not available.

The efficacy of prophylactic postoperative use of dicumarol has been high. Barker *et al* (4) reported that dicumarol had been given to 1,302 patients as a prophylactic measure to prevent thrombosis and embolism Of these, 143 had had thrombosis or embolism at some time prior to the immediate operation No pulmonary embolisms developed in these patients and minor thrombosis developed in only 2 cases The prophylactic administration of dicumarol is begun on the third postoperative day and continued as indicated previously. Administration is continued until the patients have been ambulatory for several days and a day or so before dismissal from the hospital. Patients are not kept in bed or in the hospital longer than usual because they are receiving dicumarol.

MYOCARDIAL INFARCTION

Coronary thrombosis with myocardial infarction is one of the most important causes of heart disease in middle-aged and elderly patients Attempts to reduce the incidence of thrombo-embolic complications occurring in the course of coronary thrombosis with myocardial infarction have been partially successful The aims of anticoagulant treatment in this condition are as follows: (*1*) prevention of extension of the thrombus proximal or distal to the site of original thrombosis; (*2*) prevention of the formation of intracardiac mural thrombi; (*3*) prevention of venous thrombosis from which pulmonary emboli may originate; (*4*) prevention of peripheral arterial thrombosis and embolism in arteries already considerably affected by arteriosclerosis

Nay and Barnes (29) studied 100 cases of acute myocardial infarction. In 37 cases, 48 instances of thrombo-embolic complications occurred, some of the patients having more than one episode or type of vascular complication There were 15 instances of second myocardial infarction, 14 of pulmonary embolism, 8 of cerebrovascular occlusion, 7 of thrombophlebitis, and 4 of peripheral arterial occlusion This study provides a control group of patients with myocardial infarction that may be used for comparison with groups of patients treated with anticoagulants

Until the recent report by Wright, Marple, and Beck (38) on the use of anticoagulants in a large series of patients with myocardial infarction, similar studies which have been reported have been based

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on relatively small series of patients. Nichol and Page (30), in 1946, reported favorably on the use of dicumarol in 50 attacks of acute coronary thrombosis. Wright (36,37) and Peters, Guyther, and Brambel (33) reported favorably on a series of 76 and 50 patients, respectively, treated with dicumarol. Parker and Barker (31), using Nay and Barnes's work as a control study, evaluated the use of anticoagulants in the treatment of 50 patients with myocardial infarction. In 10 cases, combined heparin and dicumarol therapy, as described earlier, was used; in 40 cases, dicumarol alone In 2 cases, thrombo-embolic complications developed during anticoagulant therapy; in 1 of these cases there was a second myocardial infarction and in the other case there was a cerebrovascular occlusion One patient had pulmonary embolism and 1 had peripheral arterial occlusion. These latter two complications occurred before treatment with anticoagulants was begun. Although this series is small, it is evident that the incidence of thrombo-embolic complications is markedly lessened as a result of the use of anticoagulants The mortality rate in the Parker and Barker series was 10 per cent; in the Nay-Barnes series (29), 13 per cent. Thus, although there was a distinct decrease in the occurrence of thrombo-embolic episodes in the group of patients treated with anticoagulants, the mortality rate was not greatly affected Minor abnormal bleeding occurred in 3 patients receiving anticoagulants (6 per cent), but there was no serious bleeding.

Wright, Marple, and Beck (38) have reported on 800 patients who had myocardial infarctions Approximately one-half (432) of the patients received anticoagulants; the other half (368) received conventional treatment without administration of anticoagulants The two groups were considered to show "a striking similarity with regard to age, history of previous infarction and estimated severity of the present attack." This group of patients was studied by many physicians, in numerous hospitals in several cities These physicians were guided in their study by a set of principles, in order to maintain a standard form of therapy Heparin was given until there was satisfactory reduction in prothrombin as a result of administration of dicumarol, which was given from the first day The Link-Shapiro method of using undiluted whole plasma was used for determining prothrombin activity Dicumarol was given in amounts of 200 to

300 mg. daily until the prothrombin time was 30 seconds. No dicumarol was given on days when the prothrombin time was greater than 35 seconds; 50 to 100 mg. of dicumarol were given when the prothrombin time was between 30 and 35 seconds. Treatment was continued for a minimum of 30 days.

Concerning administration of dicumarol, Wright and co-workers wrote: "It is our experience that a safe and effective therapeutic range [of plasma prothrombin] is between 30 and 50 seconds by the Link-Shapiro modification of Quick's one stage method [author's note: prothrombin time determination]. This range, as interpreted in our laboratory, would approximate a prothrombin activity of between 20 and 10 per cent. For heparin, the clotting time should be approximately three times normal."

The mortality rate in the control group of patients was 24 per cent, while that of the group receiving anticoagulants was 15 per cent This was considered to be a significant decrease in mortality rate In the control group of those who died, 10 per cent suffered from one or more thrombo-embolic episodes before death, while in the treated group of those who died only 3 per cent were so afflicted Fourteen per cent of the control group and 12 per cent of the treated group died without antemortem clinical evidence of further thrombosis or of embolism. It may be seen from this that anticoagulant therapy was apparently responsible for the major portion of the decrease in mortality rate in the treated group. It is considered that this is brought about by the decrease in the incidence of thrombo-embolic complications which, directly or indirectly, terminate in death of the patient.

One or more thrombo-embolic episodes occurred in 25 per cent of the control patients and in 11 per cent of the treated group. Presumably, 5 per cent of the treated group had not received adequate lowering of the plasma prothrombin. Thus, in only 6 per cent of the adequately treated group did thrombo-embolic complications develop.

The number of thrombo-embolic complications for each 100 control patients was 36; for each 100 treated patients, 14 When cases with inadequate lowering of plasma prothrombin were excluded, this latter figure decreased to 6 5. In considering the several types of thrombo-embolic complications, the treated group showed consistently lowered rates of incidence for each type.

Abnormal bleeding occurred in 6 per cent of the control group and in 12 per cent of the treated group After analysis, it was felt that 5 per cent of hemorrhagic manifestations in the patients treated with anticoagulants were due to causes other than administration of anticoagulants; 7 per cent of treated patients, then, had abnormal bleeding due to, or increased by, anticoagulant therapy. Of 30 hemorrhages only 1 (3 per cent) was considered to be severe

It should be clear from this and other studies that anticoagulant therapy in myocardial infarction significantly lowers the mortality rate and the incidence of thrombo-embolic complications Such therapy is not attended with undue risk of hemorrhage.

ACUTE ARTERIAL OCCLUSION

An occlusion of a major peripheral artery constitutes a medical, and at times a surgical, emergency For the purposes of this chapter, suffice it to say that such occlusion may be due to arterial embolism or thrombosis Acute arterial occlusion is usually treated medically in the early phases The affected extremity is protected from all forms of trauma, and measures are taken to produce vasodilatation and to relieve pain The use of anticoagulants is begun immediately in an effort to prevent intra-arterial thrombosis in the collateral vessels and in the vessel distal to the point of occlusion In addition, if the occlusion is the result of an embolism, anticoagulant therapy reduces the possibility of subsequent embolisms We use the combined heparin-dicumarol treatment, because we desire to obtain immediate anticoagulant effect with heparin and prolonged effect with dicumarol

The efficacy of such treatment is noted in the study of 45 patients with acute arterial occlusion reported by Allen *et al.* (2). Arterial thrombosis accounted for 26 of the occlusions, while 19 were due to arterial embolism In the cases of arterial embolism in which treatment was started within 24 hours of onset of the occlusion, there was survival of the extremity in 91 per cent In those cases in which it was not possible to start treatment until after 24 hours or more had elapsed from onset of the occlusion, there was survival of the extremity in only 25 per cent In the cases of arterial thrombosis in which treatment with anticoagulants was begun within the first 24 hours, there was survival of the extremity in 81 per cent. In the

cases in which treatment was begun after a lapse of 24 hours or more, the survival rate of the extremity decreased to 50 per cent. These data disclose that the use of anticoagulants and other medical procedures is attended by a high survival rate of the affected extremities in cases of acute arterial occlusion, providing such treatment is begun early (within 24 hours of the occlusion).

We are guided in determining the advisability of embolectomy in patients with arterial embolism largely by the response to medical treatment. If, after a trial of medical treatment for several hours, the response is unsatisfactory, as evidenced by failure of the circulation of the affected extremity to show improvement, we advise embolectomy. The latter procedure should be done within 24 hours of onset of arterial embolism. If heparin has been administered, operation should be delayed until its anticoagulant effect has disappeared. This usually occurs within 2 or 3 hours after administration. If delay is inadvisable, salmine may be used to neutralize the anticoagulant effect of heparin. Actually, surgical procedures have been performed when the coagulation time of the blood has been elevated owing to administration of heparin. These procedures usually have not been attended by great bleeding, although wound hematomas have occurred. Postoperative administration of anticoagulants in combined form is carried out.

THE POSTPARTUM STATE

Thrombo-embolic complications in the postpartum period are common enough to warrant constant alertness for their possible occurrence. The three most common such complications are iliofemoral thrombophlebitis, pulmonary embolism, and thrombophlebitis in varices. These conditions are all treated in the same manner as described for each condition earlier in the chapter. The two factors present in the postpartum state which merit further discussion are the possibility of producing excessive uterine bleeding and the possible production of a decreased plasma prothrombin state in a nursing infant.

Allen and co-workers (2) have reported administration of heparin and dicumarol or of dicumarol alone to 19 postpartum patients. Treatment was begun as early as the fifth postpartum day and as early as the eleventh day after cesarean section. There was

no abnormal uterine bleeding, although the plasma prothrombin was maintained at therapeutic levels. Since this report, however, there have been 2 cases of uterine hemorrhage apparently due to anticoagulants In both of these patients the plasma prothrombin concentration was less than 10 per cent In the presence of severe uterine hemorrhage associated with dicumarol-induced prothrombin deficiency, the therapy advised is to pack the uterus and administer vitamin K to restore the prothrombin concentration to normal.

Prothrombin times were determined on the blood of 2 nursing infants whose mothers were receiving anticoagulants. Although the mothers' plasma prothrombin was in the therapeutic 10 to 30 per cent range, the infants' plasma prothrombin showed no significant decrease. Though this would tend to indicate that nursing infants do not suffer a decrease in plasma prothrombin when the mothers are receiving dicumarol, the series of 2 cases is extremely small. It is probably justifiable, indeed advisable, to administer vitamin K daily to such infants If this is not done, then the infants' prothrombin times should be determined, and if the times are prolonged, the infants should certainly receive vitamin K.

We have not administered dicumarol to pregnant women A recent report by Kraus, Perlow, and Singer (23) indicates a high incidence of fetal mortality and abnormality when pregnant rabbits are given dicumarol This does not necessarily apply to humans, but it seems justifiable to advise against use of dicumarol in pregnant women until definite information is available that such therapy is without danger

THROMBOSIS OF CENTRAL RETINAL VEINS

The experience of the consultants at the Mayo Clinic in the use of anticoagulants in thrombosis of central retinal veins has not been favorable Use of anticoagulants in this condition has not led to a better ultimate prognosis.

AURICULAR FIBRILLATION

Auricular fibrillation is sometimes associated with peripheral, cerebral, or pulmonary arterial embolisms The major therapeutic problems presented in this state are (*1*) prevention of formation of fresh thrombi, and (*2*) conversion of auricular fibrillation to a normal sinus rhythm

Anticoagulants are given in an attempt to prevent fresh thrombi and thereby to reduce the possibility of arterial embolism. However, unless the auricular fibrillation is converted to a normal sinus rhythm, anticoagulants must be administered indefinitely. Unfortunately, this is rarely possible, owing to lack in many communities of facilities for adequate laboratory control of administration of dicumarol. It may therefore be advisable to attempt a conversion of cardiac rhythm with quinidine Before instituting this procedure, we reduce the patient's plasma prothrombin to the therapeutic 10 to 30 per cent range. This is done in an effort to reduce the likelihood of occurrence of arterial embolism Clinicians disagree whether or not this is a real danger. With administration of anticoagulants beforehand, we hope we have largely circumvented this danger, although conclusive evidence is lacking If conversion to a sinus rhythm is achieved, anticoagulant therapy may be stopped in seven to ten days. We do not know when the danger of embolism is passed, but we feel that after the foregoing length of time it is safe to discontinue administration of anticoagulants. Unfortunately, the number of patients in whom our associates and we have been able to convert auricular fibrillation to a normal sinus rhythm is small. If conversion is not successful, the only alternatives are the use of anticoagulant therapy for an indefinite period or acceptance of the risk of embolism.

CONGESTIVE HEART FAILURE

Congestive heart failure, with the resultant decrease in efficiency of propulsion of blood, predisposes to thrombo-embolism, which may take the form of thrombophlebitis, arterial thrombosis, intracardiac thrombosis, cerebral thrombosis, pulmonary embolism, or peripheral arterial embolism Congestive heart failure is most common in the older age groups, and these patients are likely to have arteriosclerosis, which adds to the tendency to intra-arterial thrombosis. Pulmonary embolism is not uncommon as a cause of death in patients with congestive heart failure. It is logical to assume, therefore, that administration of anticoagulant drugs to patients *in congestive heart failure has a rational basis*

It is in conditions such as this that the inadequacy of heparin and dicumarol is most apparent, for treatment should be carried out

over long periods and neither heparin nor dicumarol is satisfactory *for this purpose. However, experience is accumulating that* after periods of very close observation of the effects of dicumarol, the physician may determine an approximate maintenance dose of dicumarol for each individual patient. In many instances this is 50 to 75 mg. a day, but it must be emphasized that there is no general maintenance amount of dicumarol After a preliminary period of study it may be possible to administer dicumarol safely by determining the prothrombin time only once a week. It should never be administered without some laboratory control.

CHRONIC OCCLUSIVE ARTERIAL DISEASE

Thromboangiitis obliterans and arteriosclerosis obliterans are chronic occlusive arterial diseases characterized by intra-arterial thrombosis, which usually occurs gradually. In about 10 per cent of such cases, arterial occlusion occurs suddenly at some time Recurrent thrombophlebitis may occur in patients with thromboangiitis obliterans

Although anticoagulants should be beneficial in the treatment of the chronic occlusive arterial diseases, therapy for an indefinite period would be required. Here again, the inadequacy of heparin and dicumarol is clearly demonstrated Theoretically, the use of dicumarol over a long period should prevent intravascular thrombosis and prevent further ischemia We do not have conclusive evidence that this can be accomplished Barker and co-workers (4) reported the use of dicumarol in 76 patients with thromboangiitis obliterans or arteriosclerosis obliterans In some cases this was given for 4 to 6 months In 40 cases, the disease was considered to be in an active phase when the dicumarol was being administered In 36 cases, dicumarol was given prophylactically after amputation. In none of these cases was any further evidence of intravenous or intra-arterial thrombosis observed

MISCELLANEOUS MEDICAL DISEASES

Polycythemia vera seems to predispose to intra-arterial and intravenous thrombosis When it does, in addition to attempts to decrease the degree of polycythemia, prevention of subsequent thrombo-embolic episodes is indicated This is best done by administering dicumarol until the polycythemia has been controlled

Malignant tumors sometimes are associated with venous thrombosis The most common offending tumors are retroperitoneal neoplasms (neoplasms of pancreas or kidneys or retroperitoneal lymphosarcoma), pelvic neoplasms, bronchogenic carcinoma, and carcinoma of the stomach The occurrence of thrombophlebitis without apparent cause in patients over 40 years old should cause the attending physician to suspect a malignant lesion Anticoagulants may be used in the manner which is most appropriate for the type of thrombo-embolic episode present in the patient with a malignant tumor.

Severe infections, such as pneumonia, brucellosis, or typhoid fever, predispose to intravascular thrombosis This is particularly true in the older age groups Similar factors probably prevail here as mentioned under congestive heart failure In addition, the severe infection may be an added factor, but the exact reason for this is unknown. Anticoagulants may be used prophylactically or therapeutically in severe infections

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Hepatitis and Cirrhosis of the Liver

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Acute Virus Hepatitis

Introduction The high incidence of infectious hepatitis in World War II presented a major medical problem which led to extensive studies Although several types of acute hepatitis previously had been recognized as entities, the investigations carried out during the past eight years have done much to clarify our knowledge of the etiology, epidemiology, clinical features, and management of these diseases The literature on this subject is too vast to cite in detail in the present review. However, in the bibliography selected, the reader can find ample references to the pertinent source material

Etiology It is believed that one or more viruses are involved, since the icterogenic agent passes through bacteria-retaining filters. The agent is relatively resistant to heat (56 C for 1 hour), to freezing, to drying, and to various disinfectants such as chlorine and merthiolate, but it is apparently inactivated by exposure to ultraviolet light Attempts to identify the virus, to propagate it in tissue culture, or to transmit the disease to laboratory animals have been unsuccessful

Most of our knowledge concerning the disease has been derived from clinical and experimental studies on human volunteers (11, 12, 13, 18, 28, 30, 31, 33, 35) In the course of this work it appeared that there were certain differences between the agent of infectious hepatitis (catarrhal jaundice) and that of homologous serum hepatitis (transfusion jaundice) The former strain was called virus IH by Neefe, and the latter virus SH.

A peculiar type of infectious hepatitis with high mortality has been recently reported from Denmark This occurred in epidemic form during 1944 and 1945, and almost exclusively in women Whether this represents a separate virus strain has not been ascer-

was believed for many years that benign infectious hepatitis was caused by catarrhal inflammation of the ampulla of Vater, whereas acute yellow atrophy was a distinct lesion resulting from massive necrosis of the liver. Although certain earlier writers had maintained that the two conditions differed only in degree and extent, it was Eppinger (10), in 1922, who clearly demonstrated the presence of hepatocellular damage in early stages of infectious hepatitis. Since over 99 per cent of patients recover from acute hepatitis, the histologic changes in the liver were known only through a few chance specimens obtained at operation or autopsy. However, in the past ten years, by means of liver biopsy technics, much information has been added concerning the developmental and reparative stages of hepatitis. In these studies, as in clinical observations, no essential differences were found between the naturally occurring epidemic hepatitis and homologous serum jaundice.

BENIGN ACUTE (VIRUS) HEPATITIS

In the studies of Roholm and Iversen (37), Dible *et al.* (9), Axenfeld and Brass (2), Mallory (29), and Lucké (27b), several steps in the progression and regression of the lesions have been described. The earliest change seen is an inflammatory cellular infiltration in the periportal areas. The inflammatory cells are predominantly histiocytes and lymphocytes. This cellular reaction extends toward the center of the lobule. Later, as individual cells show signs of degeneration, the hepatic cords become disorganized. The degenerating liver cells become eosinophilic, their nuclei pyknotic, and finally they shrink into a hyalinlike mass which is extruded into Disse's space and phagocytized by monocytes. Throughout the active phase of the disease other liver cells show signs of regeneration, such as mitotic figures and multinuclear cells. In the canaliculi near the centers of the lobules, bile thrombi are frequently seen. It is noteworthy that the supporting tissue is relatively unaffected. Similar histologic changes except for the signs of bile stasis, are seen in biopsies of patients in the preicteric stage and in nonicteric cases (those who fail to develop jaundice).

Biopsies made during the recovery stage usually show marked regression of the lesions within 3 or 4 weeks after the onset of symptoms. Regenerating liver cells assume their normal relations to

tained. It should also be mentioned that acute hepatitis occurs in two other virus diseases, namely yellow fever and infectious mononucleosis. However, only infectious (epidemic) hepatitis and homologous serum hepatitis will be discussed here.

Epidemiology. Infectious hepatitis (virus IH) is world-wide in distribution, occurs predominantly between the ages of 6 and 30, with *about equal sex frequency, and has its peak incidence in the* autumn. The transmission of infectious hepatitis is effected orally or parenterally from infected feces or serum It is essential therefore to take the same precautions with this disease that are employed in other enteric infections. Whether or not transmission can be effected by contaminated urine or nasopharyngeal droplets has *not been established. The highest infectivity is in the incubation* period of 2- to 6-weeks and in the icteric stage of the disease. Infectivity is minimal after the subsidence of jaundice Although authentic cases of multiple attacks have been recorded, the large majority of patients have prolonged immunity.

Homologous serum hepatitis (virus SH) is a man-made disease, occurring at *all* ages. It is "man-made" in the sense *that it is transmitted* by the parenteral injection of contaminated blood or blood products, such as serum, plasma, or certain vaccines combined with serum that contains the icterogenic agent The virus is present during the prolonged incubation period (2–5 months) as well as during the icteric stage of the disease. Only minute quantities of infected material are necessary to cause the disease. Indeed, *there is evidence* to suggest that improper sterilization may be the cause for many cases Because of this possibility, multiple-syringe procedures should be abandoned in clinical practice There is no conclusive evidence of indirect transmission, as by urine, feces, or nasopharyngeal washings

There is relative homologous immunity but no cross *immunity* for the IH and SH strains of the virus These observations on human volunteers are in accord with experience in the field (13). It has been observed that patients who had epidemic hepatitis were susceptible to homologous serum hepatitis, and conversely that those with homologous serum hepatitis later became susceptible to epidemic hepatitis

Pathologic Changes. Following the concept of Virchow (41), it

not be presented in detail. There is a prodromal period of 1 to 3 weeks during which fatigue, fever, nausea, headache, abdominal discomfort, and generalized aches are experienced Tenderness and enlargement of the liver are usually present early in the disease. With the appearance of jaundice fever tends to abate while nausea, malaise, and abdominal distress persist for a week or more before subsiding. Lymph node enlargement is common. Splenomegaly, urticaria, intermittent diarrhea, and pruritus occur occasionally. (Unlike obstructive jaundice, the pruritus is seldom distressing) There is a longer prodromal period in homologous serum hepatitis than in epidemic hepatitis. Fever is less conspicuous but urticaria more common in homologous serum hepatitis than in the epidemic variety.

Among the most helpful tests for early detection of acute hepatitis are the bromsulfalein dye, cephalin flocculation, and thymol turbidity tests In evaluating the course of the disease, the serum albumin, serum cholesterol partition, and serum bilirubin are of considerable value. An unfavorable trend is indicated by decreased serum albumin, decreased cholesterol ester, and progressively increased serum bilirubin.

Recovery from the acute illness usually is uneventful after a period of disability of 6 to 8 weeks. Relapses have occurred in 1 to 18 per cent of different series of cases These recurrences generally take place within 2 to 4 months of the onset. In those instances with long intervals between recurrent episodes, it seems more likely that a form of latent hepatitis may have existed or that a separate and distinct type of hepatitis may have supervened

In a small group, estimated to be about 0 2 to 0.5 per cent, there is progressive deterioration with the development of *acute yellow atrophy* (27) This occurs typically within 2 weeks of the onset of jaundice, but it may occur in the form of a sudden relapse after a few months Acute yellow atrophy cannot be predicted on the basis of initial severity of the illness, since in many instances the early symptoms have been mild. The fatal turn of events is characterized by deepening jaundice, abdominal pain, pernicious vomiting, mental confusion or torpor, hemorrhagic phenomena, and the appearance of ascites. These features, together with severe functional derangement as shown by laboratory tests, are ominous signs

ducts and blood vessels. Periportal cellular infiltration may persist considerably after restoration of liver cells A slight inflammatory reaction may be present whether or not there are clinical signs of the disease. Such changes are commonplace in patients who have a delayed recovery with persistent mild symptoms However, in those with frank relapses, the biopsy findings tend to show liver cell necrosis and disorganization as widespread as during the initial attack

Mallory (29) has made special mention of a group of patients diagnosed as "chronic hepatitis without jaundice" These patients had tender, enlarged livers but the results of laboratory tests were normal. In 10 such cases, no changes characteristic of hepatitis were found.

FATAL ACUTE HEPATITIS (ACUTE AND SUBACUTE YELLOW ATROPHY)

A rapid, downhill fatal course takes place in about 0 2 per cent of the cases of acute hepatitis The pathologic findings here have been well described in monographs by Bergstrand and Lucké (27a) In the more acute form death occurs within 10 days of the onset of symptoms The liver is usually smaller than normal, its color frequently mottled by yellow, red, and purple patches On section, massive necrosis is seen, chiefly about the central veins. The liver cells are autolyzed—only the reticulum framework remains intact Inflammatory cellular infiltration occurs in the periportal and intralobular areas Regeneration is seldom seen in this group

In subacute yellow atrophy, with death occurring in 20 to 50 days, the liver shows signs of nodular regeneration as well as necrosis Bile duct proliferation also is very prominent In some instances this may be more apparent than real, due to collapse and condensation of stroma

It has not been determined whether the massive central necrosis of acute yellow atrophy represents a distinct insult to the liver or whether it is merely an exaggeration of the spotty necrosis seen in biopsies of benign infectious hepatitis

CLINICAL FEATURES

The characteristic features of acute virus hepatitis have been fully described (3,7,8,13,15–17,20,30,38,40,43) in recent years and need

not be presented in detail. There is a prodromal period of 1 to 3 weeks during which fatigue, fever, nausea, headache, abdominal discomfort, and generalized aches are experienced. Tenderness and enlargement of the liver are usually present early in the disease With the appearance of jaundice fever tends to abate while nausea, malaise, and abdominal distress persist for a week or more before subsiding. Lymph node enlargement is common Splenomegaly, urticaria, intermittent diarrhea, and pruritus occur occasionally (Unlike obstructive jaundice, the pruritus is seldom distressing.) There is a longer prodromal period in homologous serum hepatitis than in epidemic hepatitis Fever is less conspicuous but urticaria more common in homologous serum hepatitis than in the epidemic variety.

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HEPATITIS WITHOUT JAUNDICE

In recent studies (3,8,13,15,17) it became evident that hepatitis also occurs without overt jaundice Many patients are seen during epidemics with signs and symptoms that are milder but otherwise similar to those of patients who develop jaundice. Fever, lassitude, anorexia, gastrointestinal upsets, tender enlargement of the liver are present. The serum bilirubin is either normal or slightly increased. However, bilirubinuria, bromsulfalein dye retention, urobilinogenuria, and positive flocculation tests are generally present.

It has been estimated that the subicteric and nonicteric cases equal or outnumber those with jaundice. If this is so, it may explain in part the relative immunity of persons over 30 years of age. Such persons may have experienced an unrecognized hepatitis during childhood. The recognition of subicteric forms of the disease is of practical importance for several reasons: (*1*) Spread of the disease may be due largely to these unrecognized cases (*2*) The disease is incorrectly diagnosed as a functional disorder. (*3*) Casual treatment of these patients may lead to severe, protracted derangement of the liver

CHRONIC HEPATITIS

It is not known how long the virus of infectious hepatitis remains active in the host The fact that it has not been recovered from feces, blood, or liver tissue later than one month after the onset of jaundice does not rule out the possibility of persistent activity. Until humoral or biochemical methods are devised for detecting the presence of the virus and of neutralizing antibodies this problem cannot be solved

In the large epidemic of World War II, many patients were observed to have residual symptoms and signs beyond the expected 6 to 8 week course of the disease The term "chronic hepatitis" has been used for cases with this protracted course. On the basis of clinical descriptions, several stages of the disease have been grouped together, which might better be treated separately. (*1*) relapsing acute hepatitis, (*2*) protracted convalescence without signs of liver damage; and (*3*) persistence of signs of hepatitis (chronic hepatitis).

Relapsing Acute Hepatitis In a small percentage of patients with acute hepatitis under treatment, recrudescence of symptoms

and signs occur after a period of apparent recovery. The incidence varies according to the different series reported and also according to the definition of relapse. In certain cases, this refers to a return of the initial disease syndrome with frank jaundice, whereas in others the term includes cases without symptoms but with laboratory signs of functional relapse Of 200 patients hospitalized in the Middle East Theater, Havens (17) noted a relapse in 3 (1.5%) Of 200 patients hospitalized in this country for acute hepatitis, Hoagland and Shank (20) observed relapses in 18 5 per cent The additional period of convalescence in this group varied from 3 days to 10 weeks, with an average period of 20 days In a later report on 350 cases, Kunkel, Labby, and Hoagland (26) reported relapses in 49 patients (14%) of whom 47 apparently recovered completely, while two continued to have persistent signs of hepatitis. Zimmerman and associates (45) likewise reported relapses in 43 (14%) of 295 patients

In a study of 217 patients who had recovered from acute hepatitis for periods varying from 2 months to 27 years Klatskin and Rappaport (24) reported that 21 (10%) had had relapses Of these 11 occurred within 2 years and 10 occurred later than 2 years after the initial episode In some cases it was not possible to differentiate relapses from reinfections by other icterogenic agents

It is generally believed that relapses and other forms of persistent hepatitis are prone to occur with premature resumption of activity, intercurrent illnesses, alcoholism, and malnutrition Nonetheless, a small percentage takes place while patients are receiving bed rest and "optimal therapy"

Protracted Convalescence without Signs of Liver Damage. After recovery from the acute illness a considerable number of patients have persistent symptoms, although they show few signs of hepatitis They have tender, palpable livers and they complain of weight loss, fatigue, anorexia, and abdominal discomfort On the whole, they are introspective and apprehensive about their livers In a detailed study of 20 such patients by Sherlock and Walshe (39), biochemical tests and liver biopsies revealed no significant changes These authors were of the opinion that the disability was due in large part to psychogenic factors Another study by Benjamin and Hoyt (5) likewise indicated the functional nature of the complaints

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Relapsing Acute Hepatitis In a small percentage of patients with acute hepatitis under treatment, recrudescence of symptoms

differences in experience and opinion regarding residual symptoms of acute hepatitis. The ultimate fate of these cases is not yet clear, but it seems plausible that permanent, significant liver damage will remain only in a very small percentage

RELATION OF ACUTE HEPATITIS TO CIRRHOSIS

The ultimate course of patients with signs of protracted hepatitis has been a subject of considerable interest It seems fairly certain that the majority of patients with mild, persistent symptoms, whose biopsies show moderate periportal inflammation with slight fibrosis, will recover completely. However, there remain a few who show signs of progressive deterioration and die of cirrhosis of various types. The literature on this subject is confusing chiefly because the classification of cirrhosis is not agreed upon by clinicians or pathologists (12).

Postnecrotic Cirrhosis (Healed Yellow Atrophy; Coarsely Nodular Cirrhosis). This type of cirrhosis differs from subacute yellow atrophy only in the long time interval before signs of liver failure appear Whereas subacute yellow atrophy generally is considered to run a fatal course within a few months, the identical pathologic picture has been observed in patients surviving years after the initial hepatitis The author (34) has observed 15 such patients, (7 of whom came to autopsy) who survived from 6 months to 13 years after the onset of their disease The fact that the pathology in these cases remained true to type suggests that there is no tendency for this form of cirrhosis to develop into the Laennec form of the disease

Hypertrophic Biliary Cirrhosis (Cholangiolitic Cirrhosis; Hanot's Cirrhosis) Another form of cirrhosis that is being seen more frequently than in the past is hypertrophic biliary cirrhosis In these patients intermittent fever, jaundice, pronounced hepatosplenomegaly and abdominal discomfort usually continue for years before the onset of failure Laboratory tests suggest the presence of extrahepatic biliary obstruction because increased serum cholesterol and alkaline phosphatase are found, and frequently a negative cephalin flocculation reaction The liver cells appear at first to be relatively normal, but there is fine intralobular fibrosis and cellular infiltration about the small bile ducts and portal radicles The fatal termi-

in some of these cases It seems unlikely that serious liver damage develops in these patients

Chronic Hepatitis—Persistence of Signs of Hepatitis with or without Symptoms. This group is comprised of patients with signs of liver damage persisting for months or years after the initial episode of hepatitis They may be divided into two groups: (*1*) Symptomatic, comprising patients with tender, palpable livers, lassitude, anorexia, abdominal discomfort, weight loss, and impaired liver function. (*2*) Asymptomatic, comprising patients with or without palpable livers, with minimal or no symptoms, but with impaired liver function

Impaired liver function in these patients is demonstrated by bilirubinemia, bilirubinuria, urobilinogenuria, positive flocculation or thymol turbidity tests, and by increased bromsulfalein dye retention In certain cases (1,25,26) bilirubinemia alone may persist for years, without other evidence of hepatic dysfunction.

In a study of 431 cases of acute hepatitis, Barker, Capps and Allen (4) noted that 76 had evidence of hepatitis 4 months or longer after the onset Since in 29 of these there was jaundice and might be classed as "relapsing cases," it leaves 47 (11%) in the class of chronic hepatitis

In a study of 350 cases of acute hepatitis reported by Kunkel and associates (26), 60 cases (17%) required more than 3 months of hospitalization Of these, 49 were considered as relapses and 11 (3%) as chronic hepatitis Persistent symptoms and signs were present in 4, while 7 had bilirubinemia alone without other evidence of functional change Of the total series of 350 cases, 8 patients (2 3%) did not recover completely after more than a year

In 217 cases of presumably cured hepatitis, Klatskin and Rappaport (24) found many patients with persistent symptoms, hepatomegaly, or impaired liver function. Symptoms characterized by right upper quadrant pain and fat intolerance were observed in 26 per cent; hepatomegaly, in 27 per cent, impaired liver function, in 19 per cent It is of interest that 70 per cent of the patients were seen from 5 months to 5 years after the onset of hepatitis In most instances, the residual symptoms were compatible with good health and full activity

From the various series cited, it is evident that there have been

differences in experience and opinion regarding residual symptoms of acute hepatitis. The ultimate fate of these cases is not yet clear, but it seems plausible that permanent, significant liver damage will remain only in a very small percentage.

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The ultimate course of patients with signs of protracted hepatitis has been a subject of considerable interest. It seems fairly certain that the majority of patients with mild, persistent symptoms, whose biopsies show moderate periportal inflammation with slight fibrosis, will recover completely. However, there remain a few who show signs of progressive deterioration and die of cirrhosis of various types. The literature on this subject is confusing chiefly because the classification of cirrhosis is not agreed upon by clinicians or pathologists (12).

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nation is characterized by cholemia, at times complicated by hemorrhage or ascites formation.

The increasing number of these cases in recent years has led to the *suggestion* (2,42) that they may represent a chronic form of virus hepatitis. However, the possibility remains that this is a separate entity, for the clinical syndrome and pathologic picture are quite distinct from postnecrotic cirrhosis.

Laennec's Cirrhosis. In several reported series of Laennec's cirrhosis the incidence of ancient jaundice has varied from 7 to 13 per cent However, in a more recent series reported by Watson and coworkers (44), 33 per cent of the patients gave a story of antecedent jaundice. The question therefore arose as to whether infectious hepatitis might have led to Laennec's cirrhosis in these cases Although this seems unlikely to the writer, there is no denying the possibility It is conceivable that in areas where infectious hepatitis is endemic patients with latent cirrhosis are more susceptible to intercurrent hepatitis than the general population. In reports (9, 37) on puncture biopsies of the liver, cirrhotic changes have been described as a sequel to acute hepatitis. However, from the small sections obtained it is not always possible to define these positively as Laennec's cirrhosis. The cases have been few and the likelihood exists in certain instances that cirrhosis may have antedated the episode of acute hepatitis. At present, the evidence that infectious hepatitis may lead to Laennec's cirrhosis seems to be insufficient.

TREATMENT OF ACUTE HEPATITIS

Rest in bed is essential during the acute stage This generally requires 3 to 4 weeks Even in the period of convalescence periodic rest during the day is desirable, since exercise may cause persistence or exacerbations of the disease (3,18). Gradual resumption of activity, therefore, is indicated

The dietary management of these patients has been a controversial subject Whereas severe restriction of fat and protein was formerly in vogue, the present trend (3,17,20,21,23) favors a diet ample in protein and only moderately restricted (if at all) in fat. A satisfactory regimen includes about 125 to 150 Gm. of protein, 350 to 400 Gm. of carbohydrate, and 75 to 150 Gm of fat daily. Emphasis should be placed on the palatability of the food, since

anorexia may be profound. If the patient refuses to take solid food liquid diets may be given, containing milk (whole or skimmed), milk powder, eggs, and brewers' yeast powder. Such diets also can be given by tube For those who are unable to take food by mouth, intravenous infusions of glucose solution are indicated These probably should be supplemented with nicotinamide, thiamine chloride, and ascorbic acid. In patients who are edematous and who have low serum albumin concentration, the use of mercurial diuretics, restriction of sodium, and the administration of concentrated human albumin solution may be quite efficacious. There is no convincing evidence, however, that choline, cystine, methionine, inositol, liver extract, or protein hydrolysates exert beneficial effects in this type of liver disease.

Since infectious (epidemic) hepatitis probably spreads chiefly by the oral route, rigid precautions should be taken against fecal contamination During epidemics it is desirable to administer gamma globulin to exposed persons (14,19), this has been shown to exert a protective effect during the incubation period in some epidemics However, gamma globulin is ineffective after the prodromal or icteric stage of the disease has begun It also is said to be ineffective in preventing homologous serum hepatitis

Laennec's Cirrhosis

ETIOLOGY

Clinical Observations Cirrhosis of the liver is a chronic inflammation characterized by liver cell degeneration, proliferation of connective tissue, fatty infiltration, regeneration of liver cells, and an inflammatory cellular reaction There are a large number of agents (25) which are capable of producing these changes, varying in degree and intensity This is to be expected, since the liver, like any other organ or tissue, can react to injury only in a limited number of ways A variety of agents may therefore bring about a similar pathologic picture

In the case of Laennec's cirrhosis, the most prevalent type of cirrhosis encountered in clinical medicine, the etiologic agent or agents have remained unknown Reports on this suject in recent years suggest that the disease may be related to nutritional deficiency. Attention was first directed to the possible contributory role

of dietary factors in those countries where alcoholism is an unimportant factor in the background of the disease Thus, in a report on 84 cases of cirrhosis of the liver in China, Yang (48) in 1928 stated that over 70 per cent were in the laboring class who lived on coarse carbohydrate foods In 1933 Rao (35) observed that in Southern India the diets of patients with cirrhosis were usually deficient in protein, fat, and vitamins, especially vitamins A, C, and D A study of patients in Syria by Yenikomshian (49) in 1934 revealed that the diet had been poor in proteins and vitamin A, with bread and legumes as the staple foods In 1937, Tyagaraja (45) noted that the diet of patients with cirrhosis in Ceylon had been lacking in nitrogenous foods Although these authors stressed the poor dietary in the background of cirrhosis of the liver, other factors such as enteric fevers, malaria, and parasitic infestations were considered by them to be of primary etiologic importance It should be noted that these diseases would aggravate a state of nutritional deficiency

In the Western Hemisphere, more than 50 per cent of patients with Laennec's cirrhosis are chronic alcoholics, the large majority of whom show signs of specific malnutrition (39). The frequency of dietary deficiency in these patients suggested that there might be an analogy between the occurrence of Laennec's cirrhosis and that of "alcoholic" beriberi or "alcoholic" pellagra It seemed possible that malnutrition itself might play an etiologic role. The prevalence of cirrhosis in countries where nutritional deficiencies were endemic added further weight to this hypothesis. With this in mind, therapeutic trial was made of a diet rich in protein, and supplemented with vitamin B complex (27) The improvement in these patients appeared to exceed chance expectations Subsequent reports during the past 10 years have tended to confirm these observations

Experimental Observations Studies on laboratory animals by a number of different workers have firmly established the concept that dietary factors play a significant part in the etiology of cirrhosis of the liver In 1938, Chaikoff, Connor, and Biskind (10) described the occurrence of fatty liver and cirrhosis in depancreatized dogs maintained on insulin for 2 to 5 years In later experiments (9) on normal dogs they reported the production of cirrhosis in animals fed lard and lean meat for 5 months to 1 year Although the changes were attributed to the high-fat diet, it is noteworthy that this diet was relatively low in protein

Later studies by György and Goldblatt (17) described the occasional development of liver damage in rats on a diet containing 18 per cent casein but deficient in the vitamin B_2 complex Of 300 rats, 48 showed some parenchymal changes, 29 showed necrosis, and 4 showed cirrhosis. Rich and Hamilton (37) likewise observed cirrhotic changes in rabbits maintained on diets deficient in the vitamin B_2 complex.

At about this time attention focused on the reports of another group of studies on the fatty liver (The fatty liver was of particular interest since it is associated so often with the development of cirrhosis) In 1932 and 1933 Best and co-workers (4,5) found that choline prevented fatty liver in diabetic dogs and also in rats fed excess fat In 1935, they noted (6) that casein exerted a similar effect. In 1937, Tucker and Eckstein (44) discovered that the amino acid, methionine, was "lipotropic," and was probably responsible for this action of casein and other proteins as well Later, du Vigneaud *and associates* (13,14) *demonstrated that there was a reversible* reaction between methionine and choline Given methionine the animal was able to synthesize choline and thus protect itself against fatty liver, conversely, choline can act as a donor of methyl groups in the synthesis of methionine from homocystine

With these studies in mind, several workers suspected that diets low in choline (or methionine) might lead not only to fatty liver but to cirrhosis as well This hypothesis proved to be correct Almost simultaneously Webster (47), Gyorgy and Goldblatt (18), Blumberg and McCollum (7), Daft and associates (11) reported experiments in which low-casein diets (less than 10%) produced cirrhosis in the rat. The fat content of the diet bore little relation to the experimental cirrhosis, whereas the addition of casein, methionine, or choline prevented the development of cirrhosis

More recent observations indicate that the problem is more complex than was at first thought, and that the changes in the liver are probably the result of more than a single deficiency In the early experiments it was believed that hemorrhagic necrosis, fatty liver, and cirrhosis were all part of the same process It now seems likely that these diets were low in cystine as well as in lipotropic factors Whereas choline prevents the development of fatty liver and cirrhosis, it does not prevent necrosis Necrosis, however, is prevented by methionine or casein (which are potential sources for both choline and cystine).

What relation the factor responsible for necrosis bears to cirrhosis is not clear The studies of Daft, Sebrell, and Lillie (12) suggest that necrosis has no direct bearing on the development of cirrhosis By means of a diet in which yeast was the only source of protein, Himsworth and Glynn (19) in England produced "massive necrosis" of the liver of rats in 40 to 100 days, the lesion resembling healed yellow atrophy, with areas of condensation of stroma rather than diffuse fibrosis The lesion was not associated with increased fat It was prevented by casein or methionine but not by cystine or choline. Their findings have not been confirmed by the American workers, but further studies will doubtless clear up the discrepancy. However, it is manifest that at least two dietary factors contained in casein are essential for the integrity of the liver.

How do experimental studies find their counterpart in human disease? Perhaps the most suggestive evidence that human and experimental cirrhosis are related is obtained in reports by Gillman and associates (16) from South Africa These workers observed the high incidence of cirrhosis in the Bantu natives subsisting on mealie pap and sour milk Young rats were reared on the same diet and in a significant percentage of animals cirrhosis of the liver (although somewhat atypical) developed

If there is a close analogy between experimental dietary cirrhosis and human Laennec's cirrhosis, why does not the administration of choline or methionine bring about dramatic therapeutic effects in clinical practice? Some observers, indeed, state that these substances are highly effective. However, in the experience of others, the results have been unimpressive There are at least two possible explanations why these substances may not be effective: (*1*) In cases with recovery it is possible that a nutritious diet contains optimal amounts of these substances without the need of further adjuvants (*2*) In cases of failure it is possible that they are administered at a time when changes are too advanced to respond to any therapeutic agent, however specific it may be

Comments on the Pathogenesis of Cirrhosis The above data indicate that choline is an important factor in preventing fatty liver and experimental dietary cirrhosis However, the mechanism of the development of the lesion, or its pathogenesis, is not clearly defined. Presumably, in choline deficiency the fatty liver is due to

impaired transport of fatty acids in the form of lecithin, or phosphatidyl choline, from the liver to the depots Whether the prolonged accumulation of fat in the liver alone is responsible for the development of cirrhosis remains to be demonstrated Fatty livers of long duration have been produced by other means without leading to cirrhosis This suggests the possibility that in choline deficiency some mechanism in addition to the accumulation of fat may be involved in the pathogenesis of cirrhosis

DIETARY TREATMENT

The treatment of Laennec's cirrhosis by means of diet rich in protein and supplemented by concentrates of the vitamin B complex has been the subject of a number of reports (27,28,29,31). In general, the results have indicated a more favorable response to therapy than was experienced previously

The following discussion is based on a recent report (32) on a 10 year study carried out at the Research Service (Columbia Division) of the Goldwater Memorial Hospital, New York City

In all, 124 patients with Laennec's cirrhosis in failure were treated The term "failure" was applied to those who had recent hematemesis, or who showed signs of ascites and jaundice in addition to other evidence of liver insufficiency, such as wasting, fever, gastrointestinal disturbances, and laboratory tests indicating hepatic dysfunction The chief symptoms and signs and their incidence are listed in Tables I and II, together with a similar tabulation of 386 cases of cirrhosis of the liver which served as a control group (36) The latter group comprised the case material of 5 New York hospitals during the years 1920 to 1940

Emphasis was placed upon the diet, which consisted chiefly of meat, milk, eggs, fruit, and green vegetables Eggs were served with breakfast, meat, fish, or poultry were served at 2 meals, milk was served 3 times with meals and twice between meals with 25 Gm of powdered brewers' yeast in the form of a milk nog On analysis the diet contained approximately 140 Gm of protein, 365 Gm of carbohydrate, and 175 Gm of fat—a total of about 3,500 calories

For patients who did not tolerate brewers' yeast, a crude liquid vitamin B complex was substituted Thiamine chloride (5 mg daily) and unconcentrated liver extract (5 cc twice weekly) were

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administered intramuscularly. In patients with ascites, salt was moderately restricted and fluids were limited to 2,000 to 2,500 cc daily. In certain patients with stubbornly recurring ascites the sodium intake was curtailed to about 2 Gm daily. Mercupurin or mercuhydrin (2 cc) injections were given twice weekly, combined with the daily oral administration of 4 to 6 Gm. of ammonium chloride in enteric-coated tablets. The arts of nursing and dietetics were challenged by the problem of feeding patients to whom food was usually distasteful Much patience and encouragement were needed to obtain the patients' cooperation

Results of Treatment. Of 124 patients who entered the hospital with signs of liver failure, 61 showed evidence of clinical improvement Their improvement satisfied three criteria · (*1*) disappearance of jaundice, ascites, and edema; (*2*) gain in weight and strength; (*3*) improvement in the results of various liver function tests. They were further classified as *recovered* and as *partially improved* The term "recovered" was applied to 43 patients who regained sufficient bodily vigor to resume normal activities; 3 patients in this group enjoyed good health for 5 years but later died after a relapse of the disease; 4 others died of illnesses unrelated to cirrhosis

The term "partially improved" was applied to 18 patients whose jaundice, ascites, and edema completely disappeared, but whose over-all improvement was inadequate for one of three reasons (*1*) failure to regain normal bodily vigor, (*2*) liver function tests showed only slight improvement after therapy, (*3*) improvement was not maintained Of these patients, 11 survived from 2 to 5 years after entry to the hospital

Perhaps the most convincing evidence for the efficacy of dietary treatment was derived from 12 patients who responsed satisfactorily to therapy 2 or more times after having suffered repeated relapses Characteristically, they fell into their former habits of alcoholism and grossly poor diets In almost every instance, relapses were attributable to the patient's failure to continue with the prescribed dietary treatment after discharge from the hospital In effect, they reproduced the conditions of the experiment The fact that these 12 patients responded well to the same treatment on 2 or more occasions suggests that improvement resulted from therapy and that it was not "spontaneous"

TABLE I

Clinical Data on 124 Patients with Cirrhosis of the Liver (32)

	Treated series (124 cases) Av age, 48 8 yrs		Control series (386 cases) Av age, 50 yrs	
	Number	Per cent	Number	Per cent
Males	81	65	267	69
Females	43	35	119	31
Antecedent factors				
Alcoholism	95	77	207	54
Malnutrition	91	73	67	17
Syphilis	26	21	62	16
Arsphenamine therapy	19	15	34	9
Ancient jaundice	5	4	25	6
Symptoms				
Weight loss*	88	89	206	53
Anorexia	97	78	135	38
Vomiting	63	51	115	30
Abdominal pain	62	50	121	31
Epistaxes	50	40	70	18
Hematemesis	42	36	106	27
Signs				
Ascites	115	93	301	78
Palpable liver	98	79	291	75
Peripheral edema	86	69	236	61
Jaundice	83	67	252	65
Vascular spiders	77	62	58	15
Dilated veins	76	61	91	24
Palpable spleen	69	55	170	44
Fever	61	49	93	24
Esophageal varices†	59	48	—	—

* Data obtained on 99 cases

† Includes cases of hematemesis and those demonstrated by roentgenography or at autopsy.

TABLE II

Initial Laboratory Data on 124 Patients with Cirrhosis of the Liver (32)

Test results		Number of cases	Per cent
Bromsulfalein dye retention > 10% at 30 min *		118	100
Positive Takata-Ara or cephalin flocculation test†		109	94
Serum albumin < 3 5 Gm /100 cc		104	90
Increased serum bilirubin		47	38
Average initial serum albumin	2 8 Gm /100 cc.		
Average initial serum globulin	3 6 Gm /100 cc		
Average bromsulfalein dye retention in 30 min	49% (4 9 mg /100 cc)		

* Data obtained on 118 cases, dosage, 5 mg /Kg body weight

† Data obtained on 116 cases.

administered intramuscularly. In patients with ascites, salt was moderately restricted and fluids were limited to 2,000 to 2,500 cc. daily. In certain patients with stubbornly recurring ascites the sodium intake was curtailed to about 2 Gm daily. Mercupurin or mercuhydrin (2 cc) injections were given twice weekly, combined with the daily oral administration of 4 to 6 Gm of ammonium chloride in enteric-coated tablets The arts of nursing and dietetics were challenged by the problem of feeding patients to whom food was usually distasteful Much patience and encouragement were needed to obtain the patients' cooperation

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Signs of Improvement In general, it required several months of therapy before significant changes occurred. The first intimations of improvement were gain in appetite and the feeling of general well-being. More objective changes took place later, namely the subsidence of fever and disappearance of jaundice, ascites, and edema. Figure 1 shows the disappearance of these four signs of liver

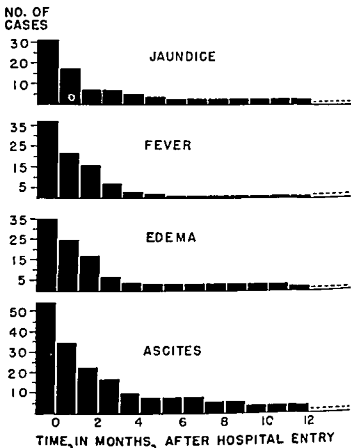


Fig 1 Length of time for signs of liver failure to disappear in 61 patients with Laennec's cirrhosis under dietary treatment (32).

failure in 61 patients who gave evidence of clinical improvement The figure portrays the duration of the signs after entry to the hospital, and does not include the prehospital period (usually months) during which the signs were present. Although there was

individual variation, jaundice and fever tended to disappear more rapidly than ascites and edema. In most instances, the signs disappeared within 3 months

Hematemesis. Presumably from esophageal varices, hematemesis occurred in 42 cases, and was fatal in 22 of them. Although approximately 50 per cent of the patients died within a year of the first hemorrhage, some patients survived hematemesis for a long time. In the present series, 9 have survived 5 years or longer without recurrence of hematemesis

Cholemia This may be defined as a stuporous state associated with and presumably due to failure of the liver It frequently is precipitated by infection or hemorrhage In the past, this picture of mental fogginess deepening into stupor and coma almost invariably was a prelude to death Yet, in the present series, 3 patients recovered from stupor and 3 from coma and they survived for periods of months or years thereafter In 4 other patients responses of several days to weeks were followed by fatal relapses. However, in 19 instances of cholemia therapy was of no avail.

Our experience tends to confirm the observations of Snell and Butt (41), who reported that dramatic recovery from cholemia may be achieved in certain cases by infusions of glucose supplemented with large doses of thiamine chloride and nicotinamide. That intravenous glucose alone may have been an important factor is suggested by earlier observations by Jones (22)

Laboratory Tests In general, the correlation between results of laboratory tests and the clinical course of the disease was only fair There may be considerable lag before test results reflect clinical changes. Indeed, in certain tests, such as the bromsulfalein dye test, results may remain unaltered for years despite sustained clinical recovery In our experience, the values of serum albumin have corresponded more closely to the clinical changes than other tests employed, and thus have served as a fairly dependable guide to prognosis (33) When the serum albumin increased to over 4 0 Gm per hundred cubic centimeters and remained at that level, the patient showed other signs of recovery The inability to achieve this value was seen usually in patients who failed or who made only partial improvement The mean value for serum albumin before therapy

was 2.8 Gm per hundred cubic centimeters, and 4.2 Gm. after therapy in the 61 patients who improved.

Changes in serum globulin were erratic and consequently less helpful as laboratory aids. The mean initial value was 3.6 Gm. per hundred cubic centimeters; after therapy, the mean value was 3.2 Gm. in the 61 patients who improved.

In all instances, the initial bromsulfalein test showed increased dye retention. In 40 of the 61 patients with improvement there was a significant decrease in dye retention, and in 9 of them the dye retention fell to within normal limits after therapy (less than 10% at ½ hour). In 21 cases there was a slight decrease or no decrease in dye retention, despite other signs indicating improvement.

Initial Takata-Ara or cephalin flocculation tests were positive in 53 of the 61 patients. In 5 instances, the tests were negative at entry, and in 3 others the data were inconclusive. In 21 instances these tests became negative coincident with improvement.

Other tests, such as serum vitamin A and carotene, intravenous galactose tolerance test, urine urobilinogen excretion, serum cholesterol and cholesterol esters, and plasma prothrombin time were performed on selected groups of patients. These liver function tests also reflected in a crude fashion the clinical status of the disease.

Pathologic Changes. There was poor correlation between the clinical course of cirrhosis and the gross or microscopic findings in the liver. The limited data obtained from this series suggest that clinical recovery seldom is accompanied by significant anatomic changes.

Failure of Therapy. Of 124 patients who entered with signs of liver failure 54 showed no response to therapy. In many instances they were too ill to cooperate in the dietary program. It is possible that some of these patients had too little functioning liver tissue to respond to any mode of therapy. Seven patients died within 2 weeks and 23 died within 1 month of entry.

In certain cases intercurrent infection or hemorrhage from ruptured esophageal varices proved to be more than these debilitated patients could withstand. There were 16 who died from infection and 22 from gastrointestinal hemorrhage.

Statistical Evaluation. The period of survival of the present series was compared with that of the control series. The duration of

life from the onset of ascites formed the basis of this comparison. (A previous paper (31) described the method of computation) The greater longevity of the present series is shown by Figure 2. At the end of 1 year 65 per cent of the treated series were alive and 39 per

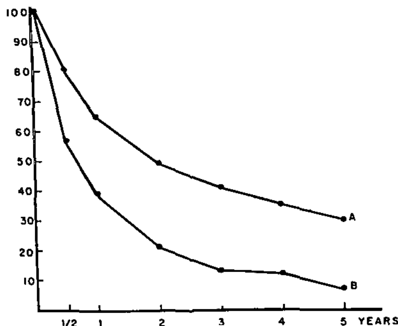


Fig 2 Survival of patients with cirrhosis of the liver from onset of ascites (32). A, Treated series of 115 patients B, Control series of 230 patients

cent of the controls, at the end of 2 years, 50 per cent of the treated series and 21 per cent of the controls, at the end of 5 years, 30 per cent of the treated series and 7 per cent of the controls The difference between the two series appears to be significant

Use of Specific Adjuvants The studies described indicate that improvement in symptoms and signs of Laennec's cirrhosis and increased period of survival are effected by a highly nutritious diet, rich in protein, and supplemented with vitamin B concentrates These findings have been substantiated in other reports (2,3,8,15,20,

24,26,34,40,42,46), some of which describe more favorable results with the added use of specific substances. An appraisal of the different therapeutic regimens is difficult because recent series of cases are not strictly comparable to those treated in the past. Modern methods of combatting shock, antibiotic drugs, and longer periods of hospitalization doubtless have favorably modified the course of the disease. Nondietary factors, therefore, may be involved In statistical comparisons it is essential that large numbers be employed and that the patient material be similar in kind This has not always been the case in recently reported series. It is self-evident that the most favorable results will be obtained when treatment is instituted at an early stage of liver failure.

Within the past few years a number of reports (3,8,26,34,40,42, 46) have suggested that the administration of choline or methionine may produce favorable effects apart from that provided by dietary means. In certain studies, patients received a high-calory, high-protein diet with supplements of yeast, liver, or synthetic vitamins for a control period of several weeks, occasionally of a few months When no improvement occurred at the end of this time choline or methionine was added. In other studies "control" patients received the standard high-protein diet with or without supplements of yeast, liver, and synthetic vitamins Test subjects were fed a similar diet but with added choline or methionine A greater survival period in the choline-methionine treated groups was attributed to the use of these substances In the writer's opinion, the experience is still too limited and the differences are not sufficiently great to warrant definite conclusions There is much variability in the time response of patients to dietary treatment alone, and it is therefore hazardous to interpret these data To determine whether there are substances which are specifically effective, the conditions must be subjected to more rigid controls than have been employed thus far

Our own experience has been limited to 8 patients whose course had become stabilized after 3 to 12 months of standard dietary treatment in the hospital They were in a state of constant, nonprogressive liver failure The added feeding of choline or methionine to these patients produced no apparent benefit On the basis of animal experiments it would seem that the field of promise for these substances would be in the fatty, precirrhotic stage of the disease.

Preliminary reports (24,34) on the use of intravenous liver extract are encouraging. Relatively large amounts of liver extract can be given intravenously when compared to the intramuscular route The sharp gain in appetite and sense of well-being enjoyed by certain patients has been impressive. Of 10 patients refractory to standard dietary care over many months, 3 appeared to have had dramatic benefit from this treatment in our hands.

The intravenous administration of concentrated serum albumin solution (1,21,23,43,30) appears to favor diuresis and the loss of ascites in certain patients, whereas it has been ineffectual in others It would seem to be a rational therapeutic measure, especially in those with low concentration of serum albumin The place of these newer adjuvants in the treatment of cirrhosis awaits further clinical trial.

SUMMARY

From the findings described in this review, it is apparent that patients with Laennec's cirrhosis are benefited by a nutritious, protein-rich diet, but it is not yet established whether certain dietary factors—such as choline, liver extract, concentrated human serum albumin solution—exert a specific effect in furthering repair and regeneration of the cirrhotic liver

The fact that Laennec's cirrhosis can be arrested at a fairly advanced stage indicates that it is not of necessity a progressive disease. With early diagnosis and institution of appropriate therapy the prognosis should become increasingly favorable

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Hepatic Tests*

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There are two theoretical reasons why one should perform a test of liver function in the clinic The first is to obtain evidence which will assist in the differential diagnosis of hepato-biliary tract disease It is obviously important and frequently urgent to determine whether the patient requires surgical or medical management The second is to detect the presence or absence of hepatic insufficiency, and if possible its extent Knowledge of the extent of hepatic insufficiency is useful in directing medical, pre operative and post operative management, and in making a prognosis Ivy and Roth (130).

Liver function tests have been used since the turn of the century as an important clinical tool However, the meaning of the term has changed considerably since the first attempt was made to measure the functions of the liver in clinical medicine In recent years, this originally awkward attempt has become articulate Many laboratory procedures have been described under this heading A discussion of their application should start, therefore, with definitions

There are very few tests measuring a function, chiefly metabolic, which is germane only to the liver (*true* liver function tests) Many other tests may measure a function the liver shares with other organs In practical use, however, information as to the functional status of the liver can be obtained by these tests Both groups of function tests determine a performance after creation of artificial circumstances, such as administration of a dye, drug, or food To these two groups, a third one, the largest, has to be added; this is not based on the measurement of a clearly defined function but entails the determination of biochemical, serologic, hematologic, and histologic data which may be altered in hepatic disorders. They thus provide clinical evidence of functional or structural alteration of the liver if other influences are taken into account To cover all

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tion in their clinical use. It is a challenge to build a composite picture from fragments of physiologic theories, clinical experience, and technical knowledge, which will permit practical clinical approach

Evaluation According to Physiologic Basis

For the practical description of the hepatic tests, it is easier to adhere to the orthodox, though often obsolete, enumeration according to the different substances upon which the liver acts. In an *academic listing, it would be more challenging to differentiate synthetic*, metabolic, and storage activities. However, in many tests these basic activities are not easily separated

BILE PIGMENT METABOLISM

Hemoglobin is changed, most probably by removal of the alpha methene bridge, to a green, iron-containing pigment–protein compound, verdohemoglobin, a biliverdin-iron-globin (169). After splitting off the loosely bound iron, a bilirubin-globin remains This step probably occurs in the reticulo-endothelial cells, especially those of spleen and bone marrow. This so-called bilirubin-globin migrates with albumin in the electrophoretic current and is separated by ultracentrifugation with the latter (251,314) It is thus still questionable whether it is not actually albumin The bilirubin which has been formed in the reticuloendothelial system does not pass into the urine and does not give the characteristic color with diazo reagent until after its physical properties have been changed (possibly by dissociation from the globin molecule) by treatment with alcohol, caffeine, sodium benzoate, or prolonged exposure to acids (indirect van den Bergh reaction) This characteristic behavior justifies a specific connotation as indirect-reacting bilirubin, bilirubin-globin (70), hemobilirubin (329), or bilirubin A (350). Indirect-reacting bilirubin is taken up by the liver, either by the Kupffer cells or the epithelial cells, and is secreted into the bile capillaries in the center of the liver cell cords During this passage, its characteristics are altered, probably by the Kupffer cells, in that *it shows the characteristic diazo reaction without previous* treatment (direct or prompt van den Bergh reaction) and it passes into the urine if above a threshold level in the blood The nature of

three groups derived from the original idea of measuring liver function, the term "hepatic tests" seems to be appropriate.

The number of tests available is practically unlimited since the liver, as the central organ of metabolism, influences a great many body functions and responds characteristically to the oral or parenteral administration of almost any exogenous substance The physiologist is still concerned with the problematic basis of many of these tests and the development of simpler and more specific tests. The clinician, however, has the responsibility of selecting from a large number of hepatic tests the ones most helpful in a given situation. A single hepatic test will rarely suffice since (*1*) some may yield abnormal results in conditions where altered hepatic function or structure can be excluded (biologically "false positive" tests) and (*2*) not all liver functions are simultaneously disturbed in established hepatic alterations (dissociation of liver function (198)). Consequently, the greatest problem in the practical use of hepatic tests is their classification and evaluation

Hepatic tests can be classified in several ways.

(*1*) An evaluation of the tests according to their *physiologic basis* follows the various metabolic, storage, excretory, secretory, and other functions of the liver.

(*2*) A *dynamic* evaluation enables the tests to be grouped as to their ability to record activity, capacity, or reserve of the liver following the principle used by physiologists in describing any function test.

(*3*) A *sensitivity* evaluation permits the arrangement of the tests according to the degree of functional, clinical, or structural damage necessary to obtain pathologic results

(*4*) *An evaluation of physiologic fluctuations* involves listing the tests showing changes resulting from normal dietary variations, infancy, senility, menstrual cycle, and pregnancy.

(*5*) An evaluation of the tests with reference to *morphologic or functional changes* correlates histopathologic phenomena or functional phenomena with the individual test

(*6*) An evaluation of the tests with respect to *diseases* entails listing those showing abnormal results in different stages of the various diseases.

(*7*) The *practical* evaluation of the tests enhances systematiza-

blood stream because the liver (Kupffer or "polygonal" cells) is unable to handle all of the increased load (Fig 1). The total serum bilirubin is markedly increased, but no bilirubin is found in the urine in the absence of complicating liver damage. Nevertheless, the biliary concentration of bilirubin is significantly increased and bile casts may even form in the bile capillaries An increased amount of bilirubin enters the intestine (pleiochromia of bile), resulting in more urobilinogen than normal being formed, deeply coloring the feces. More urobilinogen is reabsorbed, and although the liver takes up its share the urinary urobilinogen concentration is elevated.

In regurgitation jaundice, prompt-reacting bilirubin reaches the blood stream either directly or via the detour of the lymphatics after having passed through the liver cell or even the bile capillary The prompt-reacting bilirubin in the serum is increased and bilirubin appears in the urine If we accept the concept that the Kupffer cells transform indirect- to prompt-reacting bilirubin, the possibility should also be considered that prompt-reacting bilirubin may leak out of the Kupffer cells if they are unable to transmit it further to the adjacent liver cells Such a situation could easily arise as a result of damage of the liver cells or stasis in the bile capillaries

Complete biliary obstruction, as commonly produced by tumors or strictures, causes stasis of bile in the dilated bile capillaries with resulting regurgitation The total serum bilirubin rises later than, but far above the level of, prompt-reacting bilirubin This piling up of indirect-reacting bilirubin in the blood stream indicates a difficulty in uptake or transmission taking the form of a Kupffer cell–liver cell block (Fig 1) However, even in prolonged obstruction the total bilirubin does not generally exceed 15 mg per hundred cubic centimeters (icterus index 150) Since the kidney removes a considerable amount of the regurgitated bilirubin, an equilibrium is produced which is disturbed only when secondary hepatic or renal damage develops (297) Also, since no bilirubin enters the intestine, no urobilinogen is formed and the feces are acholic No urobilinogen is reabsorbed and consequently none appears in the urine

In *incomplete biliary obstruction,* commonly produced by gallstones, less bile is regurgitated and the bilirubin concentration of

this change is still problematic Some have considered it as a splitting of the protein association and formation of a sodium bilirubinate which upon re-entering the blood stream probably becomes loosely bound to albumin (363). Others associated the prompt van den Bergh reaction not with a chemical change in the bilirubin itself but rather with an increased velocity of the reaction caused either by the presence of catalysts (95) or by changes in the colloid state of the bilirubin (60) Exposure to bile acids is said to stabilize this latter alteration. Whatever the nature of this alteration may be, prompt-reacting bilirubin has been called sodium bilirubinate (70), cholebilirubin (329) and bilirubin B (350). Even under normal circumstances, not all of the prompt-reacting bilirubin is secreted into the bile capillary Small amounts (below the urinary threshold level) re-enter the blood stream (363), possibly directly from the Kupffer cells The bulk of the prompt-reacting bilirubin passes through the bile capillaries, then through the connecting link between these and the bile ducts (the ampulla), and finally into the bile ducts.

Through the intrahepatic and extrahepatic bile ducts, prompt-reacting bilirubin reaches the intestine. Here bacteria reduce it through various transitional stages to at least two different pigments, mesobilirubinogen and stercobilinogen, both of which may be simply referred to as urobilinogen (363) since they yield similar color reactions The bulk of the urobilinogen is excreted with the feces and represents the main fecal pigment. Bilirubin remains unaltered in the feces only in the presence of diarrhea. Normally, a considerable amount of urobilinogen is reabsorbed in the intestine Almost all of the reabsorbed amount is taken up by the liver and re-excreted into the bile, either as urobilinogen or more probably reoxidized to bilirubin (387) (enterohepatic circulation of bile pigment). A very small amount of urobilinogen escapes the liver and appears in the urine (Fig 1)

The classic concepts of retention and regurgitation jaundice (309) and direct- and indirect-reacting bilirubins (350) are still widely held, but are under attack by authoritative investigators (94,388)

Retention of bilirubin may be due to increased blood destruction (*hemolysis*) with piling up of the indirect-reacting pigment in the

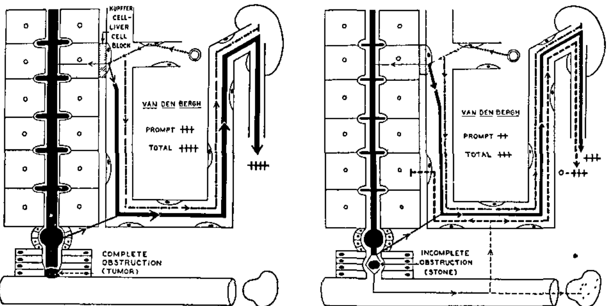
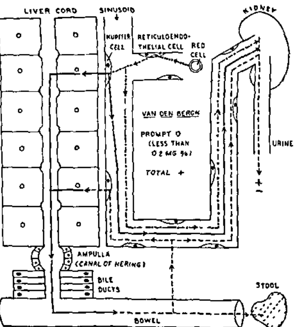
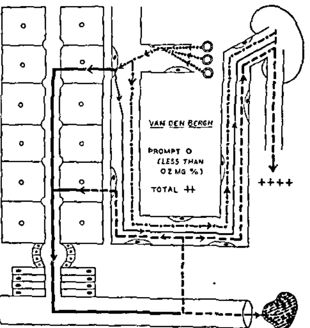


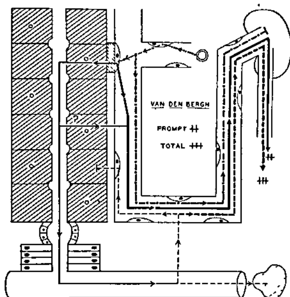
Fig 1. Schematic drawings visualizing normal bile pigment metabolism and the alterations in it in hemolytic, complete and incomplete, obstructive jaundice (redrawn and modified from Steigmann, Popper, and Meyer, 329).



Normal biliverdin globin (. ..), indirect-reacting bilirubin (-·-), prompt-reacting bilirubin (—), urobilinogen (--).

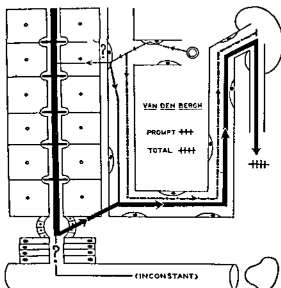


Hemolytic



Hepatocellular

Fig 2 Schematic drawings visualizing the alterations of bile pigment metabolism in parenchymal jaundice (redrawn and modified after Steigmann, Popper, and Meyer, 329) For key, see Figure 1, p 362



Hepatocanalicular

the blood and urine is not as high (Fig. 1). With a ball-valve effect of the calculus, these concentrations fluctuate. Some bilirubin enters the intestine and some urobilinogen is formed. The feces are light but not acholic. Some urobilinogen is reabsorbed but little, if any, is taken up by the liver cells due to their damage as a result of increased bile pressure (biliary hepatitis). Consequently, the urine contains urobilinogen, the amount fluctuating markedly as a result of the alternating periods of obstruction and compensatory hyperexcretion of bilirubin.

Parenchymal jaundice is due to damage of the polygonal liver cells (hepatocellular) or of the ampulla (hepatocanalicular (72)) In both types, the formation of indirect- and prompt-reacting bilirubin is normal Transmission of the prompt-reacting form into the bile capillaries is probably retarded Some bilirubin may leak back from the Kupffer cells directly into the blood stream (Fig 2), possibly due to a Kupffer cell–liver cell block In addition, it has been assumed that there is regurgitation of bilirubin in the bile capillaries through or between the damaged liver cells into the perisinusoidal space and then to the blood stream. The question whether the Kupffer cell–liver cell block or regurgitation from the bile capillary is quantitatively the more important cannot be decided Leakage *from the bile capillaries resulting from disintegration of* the liver cell cords was held responsible for the regurgitation (76) These funnel-shaped communications can rarely be demonstrated in biopsy specimens even though they are easily seen in necropsy material. Consequently, regurgitation through the liver cell cords is doubtful The elevation of the prompt-reacting bilirubin is associated with an even greater increase in the total serum bilirubin due to impaired uptake of the indirect-reacting form by the liver The increase of both prompt- and indirect-reacting bilirubin results in what has been called a delayed or biphasic qualitative reaction (366) Originally, the low ratio of prompt to total bilirubin was thought characteristic for hepatocellular lesions. However, in the milder or earlier stages, as in prodromal infectious hepatitis, the prompt-reacting bilirubin is elevated first Today, on the basis of exact quantitative tests, it is agreed that the ratio between prompt and total bilirubin is of no value in distinguishing hepatocellular from obstructive jaundice Parallel with its rise in the blood, the urinary bilirubin concentra-

BILE PIGMENTS IN BLOOD

Total Serum Bilirubin Total serum bilirubin can be determined either by comparing the color of the serum with bichromate standards (icterus index) directly (216) or after acetone precipitation (239), or else with the diazo reaction of van den Bergh (350,366) To avoid loss of bilirubin by adsorption to precipitated proteins, most newer methods use dilute ethyl or methyl alcohol. Normal values of the icterus index range from 4 to 6 units. Visible jaundice is found with values above 12 units. Marked carotenemia may produce a high icteric index The total serum bilirubin normally is 0 25 to 1.03 mg. per hundred cubic centimeters (323). Values above 2 0 mg. coincide with visible jaundice Bilirubin may also be demonstrated by a green color following the quantitative oxidation to biliverdin (168).

The level measures the degree of jaundice but is of no differential diagnostic value An excessive rise points to primary or secondary liver damage Serial determinations of total bilirubin are of greater diagnostic and prognostic value than a single analysis They permit follow-up of the disease In addition, a sudden rise indicates breakdown of hepatic parenchyma

Prompt-reacting Serum Bilirubin. The old argument as to whether prompt-reacting bilirubin (sodium bilirubinate) is normally present appears to be settled now, values up to 0 2 mg per hundred cubic centimeters are considered normal (363). The qualitative direct diazo reaction, being nonsensitive, is negative under normal circumstances The quantitative method measures the color immediately after adding the reagent. It is important not to postpone the reading beyond 1 minute after the mixing of the reagents because otherwise bilirubin-globin may be split and falsely augment the reading (366) Since this prompt-reacting fraction seems to be the first to increase in obstructive and hepatocellular jaundice, measurement of its rise may permit recognition of changes not reflected in the total bilirubin, making the test useful in the screening of prodromal and nonicteric infectious hepatitis In the presence of visible jaundice, the determination of prompt bilirubin is of little practical value It permits recognition of hemolytic jaundice, if the prompt-reacting bilirubin is not elevated in the presence of a high total serum bilirubin A ratio of direct to total bilirubin of

tion increases after the renal threshold is exceeded The threshold is high in this condition, and becomes even higher with associated renal damage Consequently, the serum bilirubin may rise high above the level seen in other types of jaundice. Despite the regurgitation, some bilirubin reaches the intestine and is transformed into urobilinogen. The feces are somewhat lighter in color than normal, and reduced amounts of urobilinogen are absorbed into the blood However, the inability of the liver cells to take it up results in an increased urinary excretion of urobilinogen

In recent years, the concept has been accepted that regurgitation of bile pigment in hepatitis is the result of backflow of bilirubin through the epithelium of the canal of Hering (363) (Fig 2). This hypothetic process has been compared to the reabsorption uremia of lower nephron nephrosis There need not be any morphologic indication of this pathologic permeability to which the term hepatocanalicular (72) jaundice has been applied. In pure forms, which are probably uncommon, normal uptake of indirect-reacting bilirubin and transmission of prompt-reacting bilirubin into the bile capillaries have to be assumed There is normal bile flow up to the ampulla, through which bile regurgitates into the perisinusoidal spaces. Marked bilirubinuria reflects the high blood concentration of direct-reacting bilirubin. Bile secretion into the duodenum is variable and may occasionally completely cease. This may result in reduced urobilinogen formation, acholic feces, and absence of urinary urobilinogen Theoretically, the van den Bergh reaction should reveal an elevated prompt-reacting bilirubin almost equal to the total However, the indirect-reacting bilirubin is also markedly elevated There are other factors which speak against permeability changes as the sole cause of this condition The occasionally observed complete absence of biliary secretion is not easily explained by the assumption of mere backflow.

In conclusion, it appears possible to adhere, in general, to the classic concepts for the explanation of most findings and for the practical diagnostic application of bile pigment metabolism, particularly if the conversion of indirect- to prompt-reacting bilirubin is assumed to take place in the Kupffer cells instead of the polygonal cells.

BILE PIGMENTS IN BLOOD

Total Serum Bilirubin. Total serum bilirubin can be determined either by comparing the color of the serum with bichromate standards (icterus index) directly (216) or after acetone precipitation (239), or else with the diazo reaction of van den Bergh (350,366). To avoid loss of bilirubin by adsorption to precipitated proteins, most newer methods use dilute ethyl or methyl alcohol Normal values of the icterus index range from 4 to 6 units Visible jaundice is found with values above 12 units Marked carotenemia may produce a high icteric index The total serum bilirubin normally is 0 25 to 1.03 mg. per hundred cubic centimeters (323). Values above 2 0 mg. coincide with visible jaundice. Bilirubin may also be demonstrated by a green color following the quantitative oxidation to biliverdin (168).

The level measures the degree of jaundice but is of no differential diagnostic value An excessive rise points to primary or secondary liver damage. Serial determinations of total bilirubin are of greater diagnostic and prognostic value than a single analysis. They permit follow-up of the disease In addition, a sudden rise indicates breakdown of hepatic parenchyma.

Prompt-reacting Serum Bilirubin. The old argument as to whether prompt-reacting bilirubin (sodium bilirubinate) is normally present appears to be settled now, values up to 0 2 mg. per hundred cubic centimeters are considered normal (363). The qualitative direct diazo reaction, being nonsensitive, is negative under normal circumstances The quantitative method measures the color immediately after adding the reagent. It is important not to postpone the reading beyond 1 minute after the mixing of the reagents because otherwise bilirubin-globin may be split and falsely augment the reading (366) Since this prompt-reacting fraction seems to be the first to increase in obstructive and hepatocellular jaundice, measurement of its rise may permit recognition of changes not reflected in the total bilirubin, making the test useful in the screening of prodromal and nonicteric infectious hepatitis In the presence of visible jaundice, the determination of prompt bilirubin is of little practical value It permits recognition of hemolytic jaundice, if the prompt-reacting bilirubin is not elevated in the presence of a high total serum bilirubin A ratio of direct to total bilirubin of

less than 40 per cent is said to exclude the possibility of obstructive jaundice (94). This notion, however, has no basis in the light of more recent experience

Indirect-reacting Bilirubin. Indirect bilirubin is determined qualitatively (350) or quantitatively by subtracting the prompt from the total bilirubin (366). A delayed direct reaction may be observed if the serum diazo reagent mixture is kept for 10 or more minutes The acid present in this mixture partially hydrolyzes the bilirubin-globin association, freeing some of the "indirect bilirubin" which then reacts as prompt bilirubin. In other words, a delayed reaction constitutes an incomplete indirect reaction Similarly, the biphasic reaction represents a partially indirect reaction Applying the same principle in the quantitative test, readings are taken after 15 and 30 minutes However, a 15 or 30 minute value represents the summation of the prompt-reacting and parts of the indirect-reacting bilirubin, but never the total The only useful division from a practical, as well as theoretic, point of view is that into the prompt or 1 minute value and total value (366).

Serum Biliverdin Recently, biliverdin was recognized as an intermediary product between hemoglobin and bilirubin, being closely related to the green hemoglobins (hemochromogens or biliverdin-iron-protein complexes) (169). Biliverdinemia is absent in hemolytic jaundice, but present in regurgitation jaundice, especially in neoplastic obstruction and also in nutritional deficiencies (166). Simplification of its analysis may give it practical significance

Bilirubin Tolerance Test The response to administration of exogenous bilirubin is considered a test for the functional reserve of the liver (356) It tests the ability of the Kupffer cells to take up bilirubin and to transmit it to the liver cells Abnormal results indicate a potential Kupffer cell–liver cell block. Bilirubin injected into the blood stream produces, as a rule, indirect bilirubinemia However, the diazo reaction of exogenous bilirubin is not established and should be the subject of further investigation Over 5 per cent retention of injected bilirubin after 4 hours indicates impaired hepatic function

The intravenous injection of nicotinic acid induces hyperbilirubinemia which subsides more slowly in the presence of hepatic damage. This can be used as an endogenous bilirubin tolerance test (323).

BILE PIGMENTS IN URINE AND FECES

Urinary Bilirubin In principle, bile in the urine indicates an elevation of the blood bilirubin above the renal threshold, for the small amounts normally present in the blood do not produce bilirubinuria This threshold seems to be raised in renal disease, in which bilirubinuria may be absent despite elevation of the blood bilirubinate concentration (79) Secondary renal lesions may also explain the lowered bilirubin excretion in parenchymal jaundice, as compared with the excretion in obstructive jaundice in the face of equal levels of sodium bilirubinate (3). A change in threshold may be responsible for the bilirubinuria present in initial stages and preicteric forms of infectious hepatitis with low bilirubinemia, while in the subsiding stage bilirubinuria is absent even in the presence of considerable blood bilirubin concentration (234). The urinary bilirubin determination may therefore serve several purposes, such as (*1*) screening for initial stages of infectious and toxic hepatitis, widely used in industrial and military medicine, (*2*) as a diagnostic procedure replacing the van den Bergh reaction for recognition of purely hemolytic jaundice, in which, despite considerable bilirubinemia, bile is absent from the urine; (*3*) for the follow-up of hepatic disease in lieu of blood bilirubin determinations, (*4*) for comparison with the urinary urobilinogen, (*5*) for the recognition of renal impairment in the presence of jaundice The qualitative demonstration or semiquantitative estimation suffices for all these purposes The older qualitative methods have been replaced by the accurate and simple paper strip test of Harrison (367) or its adaptation with tablets of plaster of Paris (81) The green color which is produced in the urine by the adding of methylene blue is of doubtful specificity for bilirubin, which does not necessarily preclude its use in screening procedures (231) Quantitative methods have not found wide acceptance because of their technical complexities Recently, a method using simple photometric determination of the diazo reaction with a correction factor for nonbilirubin chromogens has been described (92)

Urinary Urobilinogen Demonstration of urinary urobilinogen by qualitative, semiquantitative, or quantitative methods is probably the most helpful procedure in the functional diagnosis of liver disease, especially if performed serially (76,365) Reduction of urinary urobilinogen excretion points to an intrahepatic or extra-

hepatic biliary obstruction. The degree of reduction, in general, corresponds to the degree of obstruction, a relationship disturbed only by renal insufficiency and anemia. Disturbed renal function may even lead to the complete absence of urobilinogen in the urine in the presence of marked liver cell damage Elevation of urinary urobilinogen may reflect increased hemolysis or liver cell damage with subsequent interruption of the enterohepatic circulation Since the urinary concentration depends on the urinary volume, this disturbing factor can be excluded only by the use of 24 hour specimens requiring a more elaborate technic Urobilinogen reaching the blood by routes other than the enterohepatic circulation may be a source of error. For instance, bacteria in purulent cholangitis may reduce bilirubin to urobilinogen above the site of complete obstruction, or sloughed-off bile-stained epithelium may serve as a source of traces of urobilinogen. Additional errors may arise from the presence of other pigments giving the characteristic color reaction, especially porphobilinogen, or from oxidation of urobilinogen to urobilin by bacteria The qualitative test, consisting of adding Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in acid solution) should be performed on freshly voided urine. The qualitative method is helpful for orientation but may lead to erroneous results, especially since a 24 hour urine giving a negative qualitative reaction may yield as much as 15 mg of urobilinogen (365) The quantitative method (33,364) requiring collection of 24 hour urine specimens reveals normal values of 0 to 3 mg. (usually 0.5–1 5) per 24 hours (325,365). The semiquantitative method (372), using a 2 hour specimen collected between 2 and 4 p m , is sufficiently informative for routine examination Specimens obtained at this time are supposed to have a somewhat less variable excretion than at other times of the day Milligrams are replaced by Ehrlich's units, the range being somewhat similar

The diagnostic value of urobilinogen excretion depends, more than any other hepatic test, on a full appreciation of its physiologic basis; it is rarely helpful without other clinical or laboratory information. In the nonjaundiced patient, elevation in a single specimen reveals either hemolysis, or more frequently, hepatic damage interrupting the enterohepatic circulation. In this sense, it may become one of the most sensitive tests for the recognition of begin-

ning liver damage. It may indicate the degree of hepatic congestion in cardiac failure and its successful compensation In the jaundiced patient, *serial determinations with the characteristic urobilinogen* curve have become one of the greatest aids in differential diagnosis, especially if combined with estimation of bilirubin excretion (329, 365). In a malignant obstruction, there is a gradual increase in bilirubin with gradual decrease of urinary urobilinogen until it has completely disappeared (except in carcinoma of the papilla of Vater, in which fluctuations occur resulting from temporary relief of the obstruction due to sloughing of necrotic tumor tissue). In *most instances of calculous obstruction,* both urinary urobilinogen and bilirubin fluctuate. In parenchymal jaundice, toxic as well as

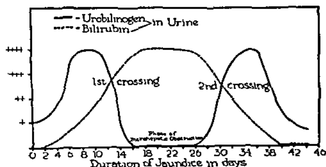


FIG. 3. Schematic curves of the results of qualitative analysis for bilirubin and urobilinogen in the urine in primary medical hepatitis with a phase of intrahepatic obstruction (329).

infections, the urobilinogen excretion is the resultant of the reduction produced by hepatocanalicular involvement (intrahepatic biliary obstruction) and the increase caused by hepatocellular damage In a typical case, urobilinogen first rises then drops while the bilirubin in the urine is rising (first crossing), with approaching recovery, the *urobilinogen may, in the face of decreasing bilirubin,* rise again (second crossing) before it returns to normal (Fig. 3) Although a full biphasic curve is rarely recognized, these crossings have prognostic significance (329)

Tolerance tests based on administration of urobilinogen (367) or stercobilin (362) are too complicated for general use

Fecal Urobilinogen and Fecal–Urinary Ratio The urobilinogen

hepatic biliary obstruction. The degree of reduction, in general, corresponds to the degree of obstruction, a relationship disturbed only by renal insufficiency and anemia. Disturbed renal function may even lead to the complete absence of urobilinogen in the urine in the presence of marked liver cell damage. Elevation of urinary urobilinogen may reflect increased hemolysis or liver cell damage with subsequent interruption of the enterohepatic circulation Since the urinary concentration depends on the urinary volume, this disturbing factor can be excluded only by the use of 24 hour specimens requiring a more elaborate technic Urobilinogen reaching the blood by routes other than the enterohepatic circulation may be a source of error For instance, bacteria in purulent cholangitis may reduce bilirubin to urobilinogen above the site of complete obstruction, or sloughed-off bile-stained epithelium may serve as a source of traces of urobilinogen Additional errors may arise from the presence of other pigments giving the characteristic color reaction, especially porphobilinogen, or from oxidation of urobilinogen to urobilin by bacteria The qualitative test, consisting of adding Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in acid solution) should be performed on freshly voided urine The qualitative method is helpful for orientation but may lead to erroneous results, especially since a 24 hour urine giving a negative qualitative reaction may yield as much as 15 mg of urobilinogen (365) The quantitative method (33,364) requiring collection of 24 hour urine specimens reveals normal values of 0 to 3 mg (usually 0 5–1 5) per 24 hours (325,365) The semiquantitative method (372), using a 2 hour specimen collected between 2 and 4 p m , is sufficiently informative for routine examination Specimens obtained at this time are supposed to have a somewhat less variable excretion than at other times of the day Milligrams are replaced by Ehrlich's units, the range being somewhat similar

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PORPHYRIN METABOLISM

The ubiquitous protoporphyrin is a hemoglobin breakdown product. Uroporphyrin, which is increased in the urine in idiopathic porphyria, is apparently the result of an "inborn error of metabolism" (87,368). Coproporphyrin, which is increased in the secondary porphyrinuria of liver disease, acts like urobilinogen in that its fecal excretion decreases when that in the urine increases (238). Two biologically occurring isomers of coproporphyrin have been found each with different properties (66). The relation in normal individuals is not constant, but in general 65 to 90 per cent of the urinary coproporphyrin is isomer I, which is apparently not a hemoglobin derivative but of unknown origin (368). The origin of isomer III, which may represent 10–35%, is also not clear, although it may possibly be derived from Hb breakdown or photosynthesis. Watson (368) states isomer I is increased in infectious hepatitis, infectious mononucleosis, extrahepatic biliary obstruction, and *nonalcoholic cirrhosis, especially the postnecrotic form III is* elevated in fatty alcoholic cirrhosis, in hepatitis due to chemical poisons, or following acute alcoholic bouts No explanation has been established for these findings. Since the bile contains isomer I, higher levels in the blood are possibly due to regurgitation They are of little value in the differential diagnosis of jaundice (368). Coproporphyrin determinations represent a very promising field for future study in liver disease

Summary. Since the determination of the two urinary coproporphyrins would permit differentiation of toxic or alcoholic liver damage from infectious hepatitis, it would have considerable practical significance. The available methods are still too elaborate for routine use.

BILE ACID METABOLISM

There is considerable support for the opinion that bile acids are formed exclusively by the liver In hepatectomized dogs, bile acids disappear from blood and urine, whereas injection of bile acids leads to their quantitative recovery from the urine (29) This would suggest an ideal test of hepatic function. Demonstration of reduced bile acid concentration in the bile is probably a rather sensitive test for hepatic impairment (224). However, this is difficult to study except in the presence of bile fistulas Since different bile acids occur in various species (318), application of animal ex-

in the stool can be measured either in milligrams in a 24 hour specimen (364) or as concentration in milligrams per 100 Gm. in a single specimen (324) The qualitative tests can indicate only complete absence of urobilinogen, as a sign of complete biliary obstruction. More conclusions can be drawn from quantitative determinations. For this, stool collection for 4 days is preferred. The excretion varies in normal individuals widely from day to day (40–280 mg), usually being 100 to 200 mg. (365) In hemolytic jaundice, it is raised tremendously, usually between 600 and 2,000 mg. per day, and up to 4,000 mg. Determination of the daily fecal urobilinogen has permitted the development of a hemolytic index (220) The ratio between fecal and urinary excretion is markedly elevated, since the increased uptake by the liver may compensate for increased intestinal absorption. In complete biliary obstruction, daily fecal urobilinogen excretion is below 5 mg., but the fecal-urinary ratio is unaltered since the urine is also free of urobilinogen Incomplete obstruction due to stones in the biliary tract seldom shows persistent values of fecal urobilinogen below 5 mg. (324) Since incomplete obstruction may be complicated by a decreased uptake of urobilinogen by the liver, the fecal–urinary ratio may be markedly depressed. In parenchymal jaundice the fecal excretion of urobilinogen is reduced, and in the presence of marked hepatocanalicular involvement may be completely absent. Since the urinary excretion is increased and the fecal excretion decreased, the fecal–urinary ratio is low. Diarrhea may reduce fecal urobilinogen A high-fat diet raises it, possibly due to a hemolytic effect of fatty acids and soaps (136) Determination of fecal urobilinogen and the fecal–urinary ratio is only occasionally of differential diagnostic help, as, for instance, in the recognition of hemolytic processes or in the separation of the antagonistic effect of intrahepatic obstruction and interruption of the enterohepatic circulation by liver cell damage

Summary. The tests in this chapter include those most helpful diagnostically, provided their physiologic basis is appreciated Thus, the demonstration of urobilinogen in the urine reflects the functional state of the liver as well as the patency of the biliary passages. The serum bilirubin primarily aids in determining the severity of the disease, whereas the bilirubin tolerance test is one of the true hepatic function tests.

mal results are seen. The degree of aberration, however, is not an index of the degree of damage (214). In earlier stages of extrahepatic biliary obstruction, the results of the test are normal. Altered intestinal absorption is a source of error in the oral test. This explains the normal values sometimes seen in cirrhosis, especially with ascites, in the presence of jaundice and severe liver damage. Extremely rapid absorption due to a sympathomimetic effect explains the increased alimentary galactose excretion in hyperthyroidism independent of hepatic damage (118)

TABLE I

Galactose Tolerance Test: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Normal subjects	162	—
Control patients	38	9 5
Acute infectious hepatitis	316	64 8
Recovering from hepatitis	13	—
Toxic hepatitis	104	83 5
Cirrhosis	229	45 4
Extrahepatic biliary obstruction	296	25 0
Tumor metastases	19	36 8
Chronic passive congestion	10	10 0
Miscellaneous	82	53 6
Total cases	*1263*	

* Shay et al. (302), Banks et al. (19), Owen (247), Rosenberg (282), Schiff en and Bockus (346), Bassett telbaum et al. (340), Colcher This summary of the litera

To overcome the alimentary factors, the urinary excretion (143) or the blood galactose (16) level are determined after the intravenous injection of 0 5 Gm per kilogram of body weight. After 75 minutes, normally no galactose is left in the blood Less than 20 mg per hundred cubic centimeters are found in recent extrahepatic biliary obstruction, while higher values usually are seen in acute hepatitis or cirrhosis. Modifications, such as a galactose index (182) or galactose removal constant (52), have been described. Results published in recent years (Table I), using various methods, indi-

periments to man requires caution The increase of bile acids in blood and urine cannot easily be interpreted because the blood cholate level represents the result of several opposing processes (138,307). Formation of bile acids is decreased in liver cell damage In regurgitation jaundice, however, blood and urine concentrations rise, due to leakage through the cholangioles Even with increasing hepatic insufficiency, the blood level in persons exposed to chemical hepatotoxic poisons does not fall, in contrast to the drop seen in animal experiments In addition, a possible enterohepatic circulation may be blocked in liver damage, preventing a re-excretion and causing a rise of the blood level As a result of this interplay of opposing factors and also of the difficulties in determinations, blood and urinary cholate levels are of limited practical value (307). They parallel roughly the bilirubin concentration, but occasionally they may not (dissociated jaundice (173)). They show no relation to the other hepatic tests (138,307). They are not changed in hemolytic jaundice. The cholic acid tolerance test offers little practical advantage (137).

Summary. Qualitative and quantitative changes of the bile acids in urine and blood are theoretically very important but they cannot be applied to practical measurement of liver function.

CARBOHYDRATE METABOLISM

Historically, probably the first function tests in clinical use measured the ability of the liver to store administered carbohydrate as glycogen

Levulose. This early test was based upon the ingestion of levulose, which in 90 per cent of patients with hepatic disease and only 10 per cent of normal individuals resulted in levulosuria (336). Since the urinary excretion depends upon renal function, only the blood levulose level is now used (333).

Galactose The original galactose tolerance test was based on determination of its urinary excretion several hours after oral ingestion of 40 Gm of galactose (17,303). Normally, almost all ingested galactose is converted into glycogen, and the urinary excretion of galactose does not exceed 3 Gm Since the results may be influenced by aberrations of the carbohydrate metabolism, such as diabetes and glycosuria, the elimination of the dextrose from the urine by yeast fermentation may be necessary Spontaneous galactose excretion is rare. In the majority of cases of parenchymatous jaundice, abnor-

Summary. The galactose tolerance test is rather helpful in the differentiation of surgical and medical jaundice in the early stages. The blood sugar level may permit recognition of severe hepatic insufficiency.

PROTEIN METABOLISM

The liver plays an important, though not fully established, role in many aspects of the protein metabolism, catabolic as well as anabolic. Amino acids administered intravenously or derived from digested protein are taken up by the liver (in addition to other organs) mainly for protein formation Some are preferentially taken up by the liver. Amino acids are deaminized in the liver, especially the alpha amino group, with the ultimate formation of urea

Serum albumin, prothrombin, and fibrinogen are apparently almost exclusively produced by the epithelial liver cells. The origin of the various globulins is problematic Chiefly due to improved physicochemical methods of analysis, such as ultracentrifugation and electrophoresis, further subdivision of the alpha, beta, and gamma globulin fractions has been accomplished (51). Alpha globulin seems to be formed by liver cells (279) Many globulins seem to be formed by the reticulo-endothelial system, of which the Kupffer cells and histiocytes in portal triads form a part. Gamma globulins increase in liver diseases, though this does not necessarily indicate their origin in the damaged liver cells They also increase in hypersensitivity states or plasma cell myelomas Specific gamma globulins, as that in serum cholinesterase and possibly complement, seem to be formed in the liver itself (53). It appears, therefore, that the liver influences the quantity of the various serum proteins and probably is responsible for qualitative changes. The liver also influences protein metabolism as a whole There is a tendency toward negative nitrogen balance in liver disease due to increased protein breakdown with the appearance of individual amino acids in the urine or increased amino acids levels in the blood.

These multiple relations between protein metabolism and liver function facilitate the clinical study of hepatic function Thus, the hepatic tests based on protein metabolism are most revealing

Total Plasma Protein The plasma protein level is an unreliable index of liver function since it is dependent on many additional factors. It is inversely related to plasma volume and there-

cate a low percentage of abnormal results in the control group, a high percentage in hepatitis, especially the toxic forms, and a relatively low one in cirrhosis and extrahepatic biliary obstruction The frequent occurrence of normal values in infectious hepatitis and cirrhosis, the variable influence of extrahepatic factors, and the lack of reproducibility of results are the reasons the original test has received little favor in recent years.

Blood Glucose. The blood sugar curve after administration of glucose is influenced by many variable factors, such as the carbohydrate and fat content of the diet, pituitary and adrenal hormones, and insulin. In addition, the level of homeostatic regulation of the blood sugar by the liver depends on the functional state of the hepatic parenchyma. In liver damage, glycogen deposition occurs at a much higher blood sugar level than normal (321) Therefore, the response of the blood sugar to ingestion of glucose is an expression of hepatic function if the other influences are taken into account (228) The glucose tolerance curve in early liver damage shows a sustained rise through the first and second hour (due to impaired glycogenesis) sometimes followed by a hypoglycemic phase. Glycosuria does not always mirror the diabetic curve. Much work would be necessary before the test could be adopted, since even in the same disease contradictory responses may be noted (348) The test has been modified by combining it with the response to insulin (173) or adrenalin (146). However, the practical value of these modifications is, at best, controversial.

Because the blood sugar drops rapidly in hepatectomized dogs (200), fasting hypoglycemia might be expected in the presence of liver damage, particularly in view of a reported defect in glycogenolysis or a lack of gluconeogenesis (53). Actually, hyperglycemia is often noted, and the blood sugar may be lowered only in severe liver damage (76). Thus, hypoglycemia serves as an alarm signal indicating diffuse, severe parenchymal involvement, such as is encountered in hepatic coma (245).

Lactic Acid The blood lactic acid level and its response to lactic acid administration have been used as hepatic tests (49). Their theoretic bases are not too clear Subsequent investigations have shown that they are not superior to the simple galactose tolerance test. The preparation of the reagent and the chemical technic is elaborate.

practical use refer to the older salting-out methods, unless otherwise stated. None of the methods in use at present for albumin-globulin partition recognize chemical differences, but are based only on molecular size and electric charge.

Albumin. Serum albumin, *supposedly exclusively produced by* the liver normally varies between 4.5 and 5 7 Gm. per 100 cubic centimeters, especially if necessary precautions for proper use of filter paper are taken (230). A drop of the albumin below this level has been found in experimental liver damage (22), and occurs in acute (119,233) as well as chronic liver disease (57,80,230) In acute hepatitis the reduction is not as marked as in cirrhosis; in the latter, the variation of the albumin level is considered one of the best indexes of hepatic activity (80,142,346).

The serum albumin concentration has been considered important in prognosis It is poor if the level is below 2 5 However, if it rises with therapy, the outlook is more favorable than when no increase or only a temporary one occurs Although serum albumin is considered an index of hepatic damage, little has been mentioned as to its value in the differential diagnosis of jaundice

Ascites formation is enhanced by low albumin levels (249). In addition, portal hypertension, protein escape through the portal capillaries, retention of sodium, and excess of the antidiuretic pituitary principle (due to deficient detoxification by the damaged liver) share in importance (197,271). However, whatever the cause, ascites reduces, in turn, the serum albumin due to its escape into the ascitic fluid Malnutrition reduces the serum albumin level, usually parallel to the globulin, whereas, in liver disease the globulin level tends to be increased.

Globulin Serum globulins vary normally between 1 3 and 3 0 Gm per hundred cubic centimeters In liver disease, the globulin rise probably does not reflect liver cell damage itself but the usually associated increased activity of the reticulo-endothelial system Thus, it may become as marked in some cases as in conditions with recognized involvement of the reticulo-endothelial system, such as Boeck's sarcoid, lymphopathia venerea, or chronic infections. The globulin increase may occasionally precede the albumin drop, but usually it parallels or follows it It may therefore also be explained

fore markedly influenced by its variations, especially if they occur rapidly. It also depends on the general nutritional status, and levels below 6 Gm or even 5 5 Gm are not uncommon in low income groups. Ascites with loss of protein into the fluid lowers the plasma protein level, especially following repeated paracenteses Finally, conditions not directly related to the liver, such as nephrosis, may have the same effect.

Principles of Albumin–Globulin Partition. The determination of the serum albumin concentration with the inherent determination of the globulin level avoids the influence of many extraneous factors. Some investigators prefer to follow the absolute albumin level, others the albumin–globulin ratio. The partition of albumin and globulin is a complex procedure, and values and ratios vary with the technic used.

The original method of albumin–globulin partition was based on a fractional salting-out of the larger protein molecule without reference to specific chemical entities The precipitate formed after half-saturation of serum with ammonium sulfate was called globulin, since it was thought to be derived from the breakdown of leukocytes The portion remaining in solution was called albumin The globulin fraction was further subdivided into euglobulins (precipitated by one-third saturation with ammonium sulfate), and pseudoglobulins. The albumin–globulin partition was later performed with 22.5 per cent sodium sulfate, permitting much better separation and the use of nitrogen determinations by Kjeldahl or nesslerization methods, which is not feasible in the presence of excess ammonium salts. The attempt is underway to base the partition on the theoretically more sound electrophoresis rather than on the original salting methods (170,203) This requires a complex apparatus and is therefore not applicable at present to routine clinical use. The albumin fraction is smaller when determined electrophorectically than by the classical salt precipitation of Howe The latter apparently does not precipitate all of the alpha globulin (203). Recently, salt precipitation methods have been developed which simulate electrophoretic partition (132,390) So far, insufficient data are available to indicate whether this newer partition will provide more practical results than the older methods patterned after half saturation with ammonium sulfate Values recorded in

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as a compensatory mechanism for maintaining colloid osmotic pressure.

Globulins were found to be elevated in experimental liver damage, hepatitis, and cirrhosis (57,230). In man, globulins are not markedly elevated in extrahepatic biliary obstruction (346). In electrophoretic studies a marked elevation of gamma globulin is noted in liver damage (98,170,203). This elevation, especially in cirrhosis (204,278), as differentiated from the smaller alpha and beta fractions, often precedes changes in the albumin–globulin ratio, and appears somewhat indicative of liver cell damage. Also, an elevation of beta globulins (probably caused by lipoproteins electrophoretically migrating with this fraction) occurs in liver disease The development of exact but simple chemical methods for determining changes of the globulin fractions in various liver diseases provides a wide field of study. Since electrophoretic determinations are too elaborate, the newer salt precipitation methods simulating the electrophoretic division of globulins are promising (132,390). Recently, a fraction precipitated by 13 5 per cent sodium sulfate was found primarily in Laennec's cirrhosis and was credited with diagnostic and prognostic significance (57).

Albumin–Globulin Ratio This is independent of variations in plasma volume. The ratio varies normally between 2 5 and 1 25. In liver disease, because of the concommitant albumin drop and globulin rise, the values are low. In practical use a ratio below 1 25 indicates hepatic damage, if other factors are excluded These may be hypoalbuminemia, e g , in glomerulonephritis, or hyperglobulinemia, e.g , in multiple myelomas, or a combination of two diseases, one causing hyperglobulinemia and the other hypoalbuminemia (140) Malnutrition influences the albumin–globulin ratio because of protein deficiency, liver cell damage, and, in an unexplained way, through the pituitary and gonads The ratio appears valuable for the recognition of hepatic insufficiency (82,142) It has been recommended for the differential diagnosis of jaundice (88). However, it should be emphasized that the ratio is an illogical figure since albumin and globulin variations follow entirely different patterns.

Flocculation Tests. In principle, information similar to that obtained from the serum protein fractionation can frequently be de-

rived from a variety of empirically devised, serologic flocculation tests. The physicochemical bases of most of these were established later by electrophoretic and other methods. The tests are therefore listed according to protein changes rather than in historic order.

Gamma Globulin Turbidity (126). The turbidity which develops if serum is diluted with an ammonium sulfate–sodium chloride solution is estimated with the standard turbidity curve The latter is calibrated by comparison with the chemical method for the determination of gamma globulin on which it is based (390). The upper limit of the normal values is 1 25 Gm. of gamma globulin per hundred cubic centimeters of serum. The results correlate well with electrophoretic determinations of gamma globulin and are independent of the albumin or lipid concentration of the serum. The gamma globulin turbidity is markedly elevated in cirrhosis, especially if associated with jaundice; less so in acute hepatitis; and barely so in extrahepatic biliary obstruction In the transition of hepatitis into cirrhosis, it may rise while the results of other hepatic tests become normal The gamma globulin elevation is considered a result of stimulation of the hepatic mesenchyma (255a)

Zinc Sulfate Turbidity Dilution of serum with solutions of metallic ions precipitates protein fractions according to the concentration used Thus, it is possible to select a concentration precipitating chiefly gamma globulin as a finely particulate suspension which can be estimated by turbidity measurement Although magnesium and cadmium sulfates have been used (391,392), zinc sulfate, in a buffer of low ionic strength, proved optimal in demonstrating this precipitation of gamma globulin (159,161). Zinc sulfate turbidity normally does not exceed 12 5 units A formula for conversion of units into grams of gamma globulin per hundred cubic centimeters has been devised (161). The turbidity is influenced by the albumin and lipid concentrations of the serum. The influence of the former is eliminated by a recent modification (177). The turbidity is elevated in various chronic inflammatory conditions (190) and in liver cell damage when associated with a rise of gamma globulin It thus permits the detection of globulin alterations in infectious hepatitis and in subsequent, persistent liver damage The highest rise is found in cirrhosis, in which the turbidity may exceed 25 units In liver damage due to extra hepatic

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biliary obstruction it is usually not elevated; this test is thus an important feature in the differential diagnosis of jaundice (191, 259,328). In conclusion, the main value of this promising test lies in the lack of elevation in the surgical type of jaundice and the excessive elevation in cirrhosis.

Colloidal Gold Test. Serums of patients with liver disease cause changes in standardized colloidal gold solutions similar to pathologic spinal fluids (96). These changes are due to increased gamma

TABLE II

*Colloidal Gold Test. Number of Cases and Percentage of Abnormal Results as Reported by Various Workers**

Diagnosis	Number of cases	Per cent abnormal results
Normal subjects	121	5 8
Control patients	409	13 0
Acute infectious hepatitis	464	77 5
Chronic infectious hepatitis	29	58 5
Toxic hepatitis	107	45 0
Cirrhosis	186	76 4
Extrahepatic biliary obstruction	152	11 8
Gallbladder disease	53	30 2
Tumor metastases	36	69 4
Chronic passive congestion	59	32 2
Miscellaneous	281	67 9
Total cases	*1727*	

* Gray (96), Mateer et al (206), Noth and Low (241), Maclagan (183–186), Shay et al (302), Neefe et al (235). This summary of the literature is not necessarily complete

globulins in both instances (97) The quantitative relationship between alpha and beta globulin on the one hand and gamma globulin on the other (20), and the serum albumin level, may have added influence (139) The preparation of the gold sol reagent itself is elaborate The buffer concentration as well as its ionic strength are important (187).

The results of the test are frequently abnormal in chronic infections not involving the liver (Table II) Abnormal results are found in about three-fourths of the cases of hepatic disease but

rarely in extrahepatic biliary obstruction The test has diagnostic value, especially since a strongly positive reaction almost excludes biliary obstruction (205) However, the complexity of its technic has deterred its wide usage.

Colloidal Red Test. In order to avoid the complications and expenses of the colloidal gold test, a similar test using colloidal red has been described (192,335) A recent modification (71) provides more easily readable results and apparently fewer "false positive" reactions than the original method. Its interpretations are similar to those of the colloidal gold test.

Formol-Gel Test The addition of neutral formalin to serum produces a gel in various diseases, e.g, in kala-azar or bacterial endocarditis This simple test for ascertaining marked globulin elevation has been recently standardized and may prove valuable as a hepatic test (23)

Takata-Ara Test. Precipitation of proteins in various serum dilutions by mercuric chloride mixed with sodium carbonate is the basis of the Takata-Ara test Elevation of the gamma globulins in addition to decreased albumin is held responsible for this precipitation (392). In general, the Takata-Ara test has little, if any, value in the differential diagnosis of jaundice (189) However, since it is abnormal far more often in cirrhosis than in acute hepatitis, it may have some value in recognizing a transition from hepatitis to cirrhosis, especially when the results become abnormal in the face of subsiding jaundice. This value is limited, since in 20 per cent of acute hepatitis abnormal results without evidence of chronic changes are seen, especially if the disease is severe (76,189) In recent years, the test has largely been discarded because the threshold for abnormal reactions is higher than that of other flocculation tests

Gros Test Hayem's solution produces precipitation when added to serum. Less is needed for this precipitation in liver disease than in normal individuals (102,196) This simple titration test is chemically related to the Takata-Ara reaction, although its exact basis is as yet unknown Results are uniformly abnormal in acute primary hepatitis, and especially cirrhosis, but also frequently so in extrahepatic biliary obstruction

Weltmann Serum Coagulation. Calcium chloride restores the heat coagulability of the proteins in diluted serum. Higher calcium chloride concentrations than normal are necessary to produce coagulation in inflammatory and exudative conditions, while lower concentrations are needed in chronic diseases with fibrotic changes (376). The test is based on alpha globulin changes, although the similarity to other flocculation tests leads one to suspect that the gamma globulins are also of some importance (290). In liver disease, especially cirrhosis, heat coagulability is restored with very low calcium chloride concentrations In hepatitis, variable results have been reported (359).

Cephalin–Cholesterol Flocculation. Serums from patients with various liver diseases are flocculated if mixed with a standardized emulsion of cephalin and cholesterol (93,106). The sensitivity of the reaction depends greatly on the age of the cephalin in the so-called cephalin–cholesterol "antigen." The fresher the cephalin, the more sensitive it is (108). Several well-standardized antigens are now commercially available. Recently, replacement of cephalin by desoxycholic acid has been recommended (330). The reaction is extremely sensitive to light (236). Whether the flocculation, which is graded 1 plus to 4 plus is read after 24 or 48 hours will depend on the sensitivity of the mixture. Usually, more attention is paid to the 24 hr. reading (235). In screening for liver damage, as in a search for infectious hepatitis, 1 plus flocculation is considered abnormal Since extrahepatic biliary obstruction may often reveal slight flocculation, 2 plus has been considered the borderline for the differentiation of intrahepatic and extrahepatic jaundice. A fractional flocculation has been devised for more quantitative estimations which may be helpful in the follow-up of acute liver disease (85) A microflocculation test using finger blood has also been devised (157).

The physicochemical basis for the cephalin–cholesterol flocculation is the relation between gamma globulin, which promotes flocculation, and albumin, which inhibits it (139). Normally, flocculation does not take place because of a balance between the two An important factor may be the loss of the inhibitory ability of the albumin under abnormal circumstances (187,223). The flocculation is not influenced by extracting the serum with lipid solvents or changing the physical state of the lipids with heparin (273).

Acute infectious hepatitis reveals abnormal cephalin–cholesterol flocculation in at least 85 per cent of the cases (Table III), even the nonicteric form. The degree of cephalin–cholesterol flocculation parallels the degree of liver cell damage (82,262,283,393). Decreasing flocculation points to improvement, while continued flocculation after the disappearance of jaundice suggests continued activity

TABLE III

Cephalin Cholesterol Flocculation Test: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Normal subjects	855	1.6
Control patients	2051	8 8
Acute infectious hepatitis	640	84.4
Chronic infectious hepatitis	110	74 4
Recovering from hepatitis	68	35 3
Toxic hepatitis	142	66.2
Cirrhosis without jaundice	128	31 3
Cirrhosis with jaundice	139	80 5
Cirrhosis	442	83 6
Extrahepatic biliary obstruction	506	20 3
Infected extrahepatic biliary obstruction	20	95 0
Gallbladder disease	174	28 2
Tumor metastases	149	52 3
Chronic passive congestion	166	54 0
Miscellaneous	439	43 2
Total cases	*6029*	

* Hanger
Soskin (283),
and Glickma

often heralding a transition to cirrhosis (95) About 35 per cent of cases of toxic hepatitis may reveal a negative reaction (255) This has been stressed in cases due to arsenotherapy (107,148,232). In cirrhosis, the percentage of cases with abnormal reactions is also high, depending on the degree of liver cell damage The greatest practical value of the cephalin–cholesterol flocculation seems to be

the differentiation between jaundice due to extrahepatic biliary obstruction and acute or chronic intrahepatic processes With the exception of one group of investigators (252), who possibly used too sensitive an antigen, all authors are agreed that flocculation is infrequent in benign or malignant extrahepatic biliary obstruction Marked liver damage caused by biliary retention finally may produce abnormal flocculation (176,281), but frequently patients dying from hepatic insufficiency caused by long-standing complete biliary obstruction with marked histologic changes of the liver do not show abnormal flocculation. The important exception to this observation is bacterial infection of the portal triads with subsequent purulent hepatitis (255,261). This phenomenon, not uncommon in calculous diseases of the biliary tract, causes a previously negative cephalin-cholesterol flocculation to become abnormal Flocculation may also be abnormal in the presence of hepatic tumor metastases and chronic passive congestion If these reservations are kept in mind, the cephalin–cholesterol flocculation is one of the most helpful tests in the differentiation of the surgical from the medical type of jaundice Abnormal flocculation, though not present in healthy individuals, has been found in bacterial endocarditis, infectious mononucleosis, nephrosis, pneumonia, and lupus erythematosus, all which may influence the serum proteins (106,291).

Thymol Turbidity Test Dilution of serum with a buffered thymol solution produces a diffuse turbidity which is frequently increased in liver disease (185). The minute precipitate formed consists of a globulin–phospholipid–thymol complex. An interplay of the following factors determines the degree of thymol turbidity: (*1*) reduction or alteration of serum albumin (162,187), (*2*) increase of gamma (162,187) and/or beta globulin (48), (*3*) increase of lipoprotein complexes electrophoretically migrating with beta globulin (145), and (*4*) increase of lipids (273), especially phospholipids (187). The importance of serum lipids in the thymol turbidity is demonstrated by the lack of turbidity after extraction of the serum with ethyl ether (273) One of the functions of the thymol in the reaction is to increase the size of the lipid globule, rendering it more visible (162) Lipemia, therefore, may falsify the results, and fasting blood should be used for the test

In various conditions and different stages of the same condition,

different factors influence the degree of turbidity. It thus appears that in early infectious hepatitis an increase of phospholipids is the primary cause of the elevated turbidity, whereas in later stages the gamma globulin elevation is more important (162).

The original visual measurement of the turbidity has been replaced by spectrophotometric readings using barium sulfate (301), Evans blue dye (163), or copper sulfate (73) as standards for the arbitrary units. Standard curves calibrated with barium sulfate suspensions are the most widely used. The original description of the preparation of the barium sulfate suspension (301) erroneously lists 0 0962 normal barium chloride solution instead of 0 0962 molar solution (73). The latter should be used to imitate the original standards of Maclagan. If this factor is not taken into account, the results based on this curve are reported twice as high as those based on the original curve of Maclagan or other modifications. Further differences are produced by variations of the barbital–sodium barbital buffer Various buffers equally prepared do not always give reproducible results (202) There is lack of uniformity in the *p*H of the buffer (lowering it from the originally recommended 7.8 to 7 5 increases the sensitivity (205) which is not necessarily desirable (184)), the temperature at which the reagent should be kept, the use of blanks, and the calibration of the standard curve. A stable alcoholic thymol stock solution and barbital buffer has been devised (126). Standardization of the procedure, and especially the calibration, should eliminate the variations in the results noted by different investigators Some consider values above 2 units abnormal, others, above 5 units (Table IV). The highest elevations are found in infectious hepatitis, they appear somewhat later than the cephalin–cholesterol flocculation, but persist longer than almost any other abnormal findings (111,162). This persistent elevation characterizes chronic, low-grade hepatitis (163). In toxic hepatitis, especially the arsenical form, normal values are not uncommon (255) In cirrhosis without jaundice the elevation is usually slight, and not as common as in cirrhosis with jaundice which may show results similar to hepatitis (202) In extrahepatic biliary obstruction, the turbidity is normal in about 75 per cent of the cases, and in the rest is rarely elevated above 7 units (186,259) However, secondary bacterial infection of the portal triads in extrahepatic biliary ob-

struction results in uniformly increased thymol turbidity values (255). Fatty metamorphosis of the liver, of itself, does not lead to elevated turbidity. Abnormal results in apparently normal individuals are rare, but are commonly seen in chronic inflammatory conditions such as rheumatoid arthritis, lymphopathia venerea, or rheumatic fever (42,160,334). The test is most useful in the differential diagnosis of jaundice and less so in the recognition of liver damage.

TABLE IV

Thymol Turbidity Test: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Normal subjects	288	2.4
Control patients	286	12 9
Acute infectious hepatitis	572	84 0
Chronic infectious hepatitis	88	72.7
Recovering from hepatitis	70	60 0
Toxic hepatitis	101	69 9
Cirrhosis without jaundice	38	71 1
Cirrhosis with jaundice	75	93 3
Cirrhosis	231	71 6
Extrahepatic biliary obstruction	271	25 8
Infected extrahepatic biliary obstruction	17	100 0
Gallbladder disease	52	15 4
Tumor metastases	45	26 6
Chronic passive congestion	46	47 8
Miscellaneous	311	35 6
Total cases	*2491*	

* Maclagan (184–186), Watson and Rappaport (371), Maizels (192), Mateer et al. (205), Shay et al (302), Mann et al (202), Neefe et al. (235), Kunkel (160), Kibrick and Clements (142), Dreyfuss (67), Stillerman (334), Popper et al. (260) This summary of the literature is not necessarily complete.

The response of the thymol turbidity to a fat meal permits its use as a simple test for fat absorption (259).

Thymol Flocculation. The flocculation developing in the thymol-buffer-serum mixture kept overnight in instances with increased turbidity has been graded either as 1 plus to 3 plus (235) or as the ratio between the original turbidity and the one reduced by flocculation (302). This thymol flocculation test does not run completely

parallel with the turbidity test. It is somewhat less sensitive and reveals more frequent "false positive" reactions. However, it is a valuable screening test, and deserves consideration in a system with other tests.

Dilution Flocculation Test. The slight turbidity produced by dilution of serum with distilled water has been recommended as a test of liver function (67). The results simulate those of the thymol turbidity test. A similar test, used extensively in the Orient in screening for kala-azar, has been called the globulin precipitation test (313).

Principle of Flocculation Tests. The factor common to all flocculation tests is an elevation of gamma globulin. This seems to be the main factor in the zinc sulfate turbidity, colloidal gold, colloidal red, and Takata-Ara tests. In other tests, such as the cephalin-cholesterol flocculation, concomitant alterations of the albumin are important. In addition, lipids and lipoproteins are important in the thymol turbidity test. The globulin increase is responsible for the diagnostically important characteristic of most of the flocculation tests; they are abnormal in the medical type and normal in the surgical type of jaundice. The globulins are, as a rule, significantly elevated in infectious hepatitis and cirrhosis, but normal in extrahepatic biliary obstruction even in the presence of severe liver damage (biliary hepatitis). Exceptions are secondary bacterial infection of the portal triads in biliary obstruction (purulent hepatitis), in which globulin elevation is seen, and, on the other hand, toxic hepatitis in which the globulins do not necessarily rise. Another factor responsible for depressed flocculations in extrahepatic biliary obstruction may be regurgitated biliary substances (probably lipids) the effect of which has been shown for the zinc sulfate turbidity (255a).

Flocculation tests do not always parallel the changes in the albumin–globulin ratio For instance, in viral hepatitis they may show abnormal results despite an almost normal albumin–globulin ratio while the albumin fraction may be reduced in biliary hepatitis by the liver cell damage resulting in a lowered albumin–globulin ratio with normal flocculation tests The globulin elevation is usually far more marked in cirrhosis than in acute hepatitis Consequently, very marked elevation of the gamma globulin and zinc sulfate turbidity and a positive Takata-Ara test may be helpful in differentiating

these two groups of conditions (328). The reverse is true of the thymol turbidity test, since the lipid changes predominate in the acute phase of hepatitis The dependence of the flocculation tests on elevation of the globulin explains the abnormal results frequently seen in nonhepatic conditions associated with reticulo-endothelial stimulation, such as chronic infections, rheumatoid arthritis, endocarditis, malaria, kala-azar, and plasma cell myeloma

The question arises whether in liver disease, the abnormal results of the flocculation tests are actually related to liver cell damage itself. Changes due to alteration of the albumin component can be expected to be so. Those due to elevation of the globulin component are more plausibly explained by stimulation of the mesenchymal elements, e g., the Kupffer cells or histiocytes in the periportal fields. The mesenchymal reaction is more marked in infectious and purulent hepatitis and cirrhosis than in biliary and toxic hepatitis Correlation of the hepatic tests with liver biopsy findings may clarify this relation.

Coordinated use of several flocculation tests in a system is of added diagnostic value (328).

Sedimentation Rate. The rate of erythrocyte sedimentation depends on the molecular size and shape, and the amount of the plasma proteins. Bile acids exert an inhibitory effect. Hyperglobulinemia increases the rate The changes of the serum proteins in liver disease are thus reflected in the sedimentation rate However, the results are not uniform. In hepatitis, the sedimentation rate is normal or even reduced in the earlier stages and elevated in later stages (119) It is usually increased in cirrhosis and surgical jaundice. Despite its nonspecific character, the sedimentation rate may be helpful under the following circumstances (other interfering factors being equal); (*1*) as an index of persistent activity in convalescent hepatitis; (*2*) for differentiation of nonicteric cirrhosis from chronic passive congestion, (*3*) in the differential diagnosis of severe jaundice, a low rate favoring parenchymal rather than extrahepatic involvement (274).

Fibrinogen. Since fibrinogen is probably formed by the liver, it may be reduced in severe parenchymal liver disease (76). However, it may be increased in cirrhosis.

Prothrombin. Hemorrhagic diathesis in liver disease has been

associated with a deficiency of prothrombin, rather than of fibrinogen (268). Prothrombin is a globulin formed exclusively by the liver under the influence of vitamin K (178). Natural vitamin K is sufficiently absorbed only in the presence of bile salts. The absence of these salts in obstructive or parenchymal liver disease causes hypoprothrombinemia Oral or preferably parenteral administration of water-soluble substances with vitamin K activity corrects a hypoprothrombinemia based solely on deficient intake or absorption However, damaged liver cells are unable to form sufficient prothrombin despite an adequate supply of vitamin K. Therefore, the response to vitamin K therapy and not the prothrombin level itself is a test of hepatic function, and one of the few examples of a true liver function test. However, it is not very sensitive and does not mirror too closely the functional state of the liver. Rarely, marked liver cell damage may be associated with a normal prothrombin response to vitamin K and relatively mild liver damage may be associated with severe uncontrollable hypoprothrombinemia The values of this very practical test therefore lies more in the prevention and control of hemorrhagic states than in the diagnosis of liver disease. Dilution of the plasma in the performance of the test is said to increase its sensitivity by magnifying the variations (7). The performance of the more complicated two stage procedure is said to increase its diagnostic value in liver diseases (201,361).

The response to synthetic vitamin K is observed after varying time intervals (395). Initial levels below 85 per cent prothrombin time or a prothrombin index (read on a standard curve) below 75 per cent are considered abnormal Following vitamin K administration, as a rule, an increase of lowered prothrombin of at least 15 per cent is considered evidence of normal or only slightly damaged liver cells. In the presence of severe jaundice, this speaks for extrahepatic biliary obstruction This rise may occur after 24 hours, whereas in parenchymal damage an immediate rise of sufficient height may be followed by a rapid drop Therefore, conclusions should be based on at least 48 hours' observation, or preferably on a maintained response This makes the systematic comparison of results difficult. An attempt has been made to standardize the test using excessive doses of vitamin K and diluted plasma for the estimation of hepatic function (347). Under these conditions, the pro-

thrombin concentration in liver damage not only fails to rise but may even drop. So far, the data presented are too incomplete to permit a definite conclusion and the explanation of this phenomenon is lacking. Much additional work, especially in standardization of the prothrombin response to vitamin K, is necessary for it to emerge as a diagnostic test.

Complement Titer. Complement, one of the serum globulins, has been associated with the function of the reticulo-endothelial system. Complement titer is reduced in liver disease (76).

Serum Nonprotein Nitrogen and Urea. Alterations in the concentration of blood urea need not parallel changes of the nonprotein nitrogen The concentrations of these substances result from the interaction of several variable factors. (*1*) Reduction of urea formation due to a defect in deaminization by the liver cells. Although low urea levels are found in hepatectomized animals (200) and in very severe hepatic insufficiency (44), as a rule, the blood urea is elevated in liver disease. (*2*) Increased nitrogen catabolism with the release of breakdown products into the blood stream, as evidenced by the negative nitrogen balance in liver disease. (*3*) Impairment of renal excretion. The urea clearance in liver disease is reduced out of proportion to the creatinine clearance (218), suggesting pathologic reabsorption of nitrogenous substances, especially urea, from the tubules, supported by the tubular damage seen morphologically.

In liver disease, renal changes outweigh deficient deaminization with its tendency to reduce urea The elevation of nonprotein nitrogen and urea is considered an index of the hepatorenal relationship Diagnostically, it is important because azotemia is generally absent in acute infectious hepatitis and only rarely found in the chronic form. It is fairly common in toxic hepatitis where, in fatal cases, a combination of cholemia and uremia is often present The determination of the nonprotein nitrogen is also of prognostic value, as an elevation points to increased liver damage in hepatitis and cirrhosis and the appearance of severe liver damage in obstructive jaundice. The mortality rate in liver disease with a nonprotein nitrogen level above 70 mg. per hundred cubic centimeters, is 78 per cent in contrast to 28 per cent when it is below 40 mg. (218).

The level of uric acid reflects the functional state of the liver,

since the liver supposedly destroys uric acid (199). Varying levels are found in liver disease. The relation between free and combined uric acid may have some diagnostic significance; the titer of the bound fraction is higher in liver disease (5). In eclampsia, there is a rise in uric acid before that of the other nitrogenous substances Whether this is related to liver damage is not known.

Amino-aciduria. In liver damage, the urinary amino acid excretion is increased. Since the determination of urinary amino nitrogen is technically difficult, more emphasis has been placed upon the demonstration of individual amino acids. The appearance of leucine and tyrosine crystals in the urinary sediment is used as a simple index of hepatic failure (173). The chemical qualitative demonstration of the phenol ring in tyrosine with Millon's reagent may also be used (76). Specific quantitative methods have been applied, such as the determination of urinary tyrosine by means of the enzyme, tyrosinase (174). Tyrosinuria occurs before the blood tyrosine level increases and was therefore considered a rather sensitive test for hepatic damage (173). Paper chromatography may be used to demonstrate individual amino acids in the urine (61). Amino-aciduria occurs in severe nutritional hepatic necrosis in animals, in so-called acute yellow atrophy, and terminally in some cases of cirrhosis (173), as well as in Fanconi syndrome (61).

Amino Acid Tolerance. After early experiments with various amino acid mixtures (76), tests using gelatin or glycine were introduced (195). Recently, protein hydrolysates of greater purity and improved biochemical methods have offered a means of studying the tolerance to the intravenous injection of amino acid mixtures In rabbits with experimental liver damage, delayed disappearance of amino acids from the blood was observed (149). However, the differences in tolerance curves or urinary excretion between normal individuals and patients with liver diseases were erratic (171,179, 332) The disappearance of amino acids from the blood does not necessarily indicate anabolic or catabolic utilization by the liver or any other organ. It may only mean storage in any organ, as is the case with intravenously administered plasma or albumin (75) The role of the liver in amino acid metabolism is therefore not reflected in tolerance tests The blood methionine level after administration of a test dose returns to normal more slowly in patients with liver

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In liver disease, renal changes outweigh deficient deaminization with its tendency to reduce urea. The elevation of nonprotein nitrogen and urea is considered an index of the hepatorenal relationship Diagnostically, it is important because azotemia is generally absent in acute infectious hepatitis and only rarely found in the chronic form. It is fairly common in toxic hepatitis where, in fatal cases, a combination of cholemia and uremia is often present The determination of the nonprotein nitrogen is also of prognostic value, as an elevation points to increased liver damage in hepatitis and cirrhosis and the appearance of severe liver damage in obstructive jaundice The mortality rate in liver disease with a nonprotein nitrogen level above 70 mg. per hundred cubic centimeters, is 78 per cent in contrast to 28 per cent when it is below 40 mg. (218).

The level of uric acid reflects the functional state of the liver,

Total Serum Cholesterol. Cholesterol is present in the bile in large amounts. Its blood level rises in extrahepatic and intrahepatic biliary obstruction from the normal of 140 to 200 mg. per hundred cubic centimeters to over 300 mg. Hypercholesteremia usually parallels the degree of jaundice in the acholic phases of arsenical hepatitis or in cholangiolitic cirrhosis. It may also occur in obesity, the last trimester of pregnancy, nephrosis, some lipoidoses, and hypothyroidism In the fulminant form of infectious hepatitis, severe or terminal cirrhosis with jaundice and toxic hepatitis, the total cholesterol level may be decreased to below 80 mg. per hundred cubic centimeters (194). The cause of this reduction is not clearly understood It has been associated with excessive fixation of cholesterol in the diseased liver (375). Reduction also occurs in fever, anemia, hyperthyroidism, and infections in general.

Serum Cholesterol Esters. Cholesterol as an alcohol may combine with various fatty acids These esters occur in organs with rather active sterol metabolism, such as liver, adrenal, and testis (74). Under normal circumstances, 60 to 75 per cent (some put it as low as 50%) of the total serum cholesterol, independent of its absolute value, is present in the ester form and the remainder is in the free alcohol form. In liver disease this ratio is decreased, somewhat parallel to the degree of liver damage (134,383) The decrease has been associated with an inability of the liver cells to esterify cholesterol though other organs possibly can do this (375). Nevertheless, the cholesterol ester ratio has been considered a valuable index of hepatic function, variations of the ratio indicating the course of the disease In cirrhosis, the incidence of abnormal results is lower than in acute hepatitis The liver damage produced by extrahepatic biliary obstruction often results in a reduction of the ratio (Table V). This makes the test less suitable for the differential diagnosis of medical and surgical jaundice Furthermore, the only accurate and reliable method (296) is too elaborate for routine use.

Fecal Fat. The increase of fecal fat in hepatic disease was previously considered to be the result of impaired absorption of fatty acids in the absence of bile acids in the intestine However, it is now known that fat absorption is not significantly reduced in the absence of bile acids On the contrary, there seems to be an excessive intestinal excretion of neutral fat as well as of fatty acids in liver disease. Fecal fat is thus not a reliable measure of hepatic function (41).

disease (145). An increased urinary excretion after methionine supplementation has been reported in patients with liver damage (105), but with a more reliable method no significant difference was found (377).

Summary. Determination of serum albumin approaches a true hepatic test since it is primarily formed in the liver. It is of particular value in chronic liver disease. The serum globulins assess the associated mesenchymal reaction. The flocculation tests, reflecting qualitative and quantitative changes in the serum proteins, are of great value in the differential diagnosis between surgical and medical jaundice, provided that possible exceptions are taken into account. Because of abnormal reactions in diseases not primarily hepatic, they are of limited value in assessing liver damage The flocculation tests are not completely identical in principle and results. Thus, it may be advantageous to perform several of them simultaneously. The prothrombin determination, representing a true hepatic test, has limited diagnostic significance Amino-aciduria (leucine and tyrosine) is helpful as an alarm signal Azotemia primarily indicates hepatorenal involvement. The tests related to protein metabolism appear to be the most helpful as well as promising

LIPID METABOLISM

With few exceptions, the blood lipids, exclusive of cholesterol, do not change in liver disease with sufficient regularity to be used as hepatic tests (134,337) Hyperlipemia occurs in xanthomatous biliary cirrhosis. The total lipids may be increased in alcoholic fatty cirrhosis (341) Neutral fats are often reduced in hepatic insufficiency and in contrast to the other lipids, are decreased in xanthomatous biliary cirrhosis. They are often elevated in extrahepatic biliary obstruction and infectious hepatitis, the levels decreasing with relief of the obstruction or subsidence of the hepatitis (194) A simple turbidity test for estimating total serum lipids has recently been devised (161) Phospholipids are, as a rule, elevated in extrahepatic biliary obstruction (returning to normal when the obstruction is relieved) and occasionally in infectious hepatitis; they are normal or even markedly reduced in portal cirrhosis, toxic hepatitis, and some cases of infectious hepatitis (194,341) Studies with radioactive phosphorus indicate increased phospholipid formation in obstructive jaundice and reduced formation in hepatic damage (11).

defect in vitamin A esterase (258) In recovery from liver disease or infections, the plasma vitamin A level rises temporarily to abnormal levels (256). In chronic liver disease such as cirrhosis, the plasma vitamin A level is exceedingly low because prolonged difficulty in absorption with subsequent depletion of the liver depots is added to the inhibition of release. The normal plasma concentrations lie between 30 and 50 μg per hundred cubic centimeters. Levels below 15 μg. are unquestionably abnormal. The response of the plasma vitamin A level after the intake of large amounts of vitamin A in oil (75,000 units) is markedly depressed in liver disease (263) However, in the diagnosis of hepatobiliary disease, neither the plasma vitamin A level nor the response to large doses of vitamin A are of much help although they may assist in the follow-up of individual cases.

Hormones There is a close relation between many hormones and the liver In particular the lack of detoxification of estrogens in the damaged liver has been emphasized (24,151) However, an improvement in methods of analysis is required before these relations will be reflected in hepatic tests

Summary The tests referring to vitamin and hormone metabolism do not as yet lend themselves to routine use.

ENZYME METABOLISM

Alkaline Phosphatase The serum concentration of this enzyme, which splits organic phosphorus compounds, is elevated in conditions with increased osteoblastic activity, such as childhood, Paget's disease, rickets, and primary and secondary bone tumors (26). In addition, it is elevated in liver disease with or without jaundice It is especially high in complete extrahepatic biliary obstruction, and only slightly elevated in hepatic insufficiency without marked interference with the bile flow (104) In the severest forms of hepatic insufficiency, phosphatase may drop. Two main hypotheses are offered for the increase of alkaline phosphatase in liver disease (*1*) Since it is normally found in the bile, deficient clearance of serum alkaline phosphatase, due to interference with bile flow, was considered responsible The faulty clearance, in turn, was explained either by a piling-up of the enzyme in the liver cells or by its regurgitation from the bile capillaries into the blood (309,360) (*2*) It has been assumed that the liver cells form alkaline phosphatase

Summary. Total cholesterol is a valuable test for biliary obstruction, whereas the cholesterol ester ratio is a measure of the degree of hepatocellular involvement, even in the presence of extrahepatic biliary obstruction.

TABLE V

Cholesterol Ester Ratio: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Control patients	144	4.2
Acute infectious hepatitis	383	80.3
Chronic infectious hepatitis	31	93.0
Toxic hepatitis	86	75.6
Cirrhosis	295	62.0
Extrahepatic biliary obstruction	285	41.3
Gallbladder disease	168	22.6
Tumor metastases	48	56.3
Chronic passive congestion	77	41.6
Miscellaneous	89	19.1
Total cases	*1606*	

* Epstein and Greenspan (77), White et al. (380), Gray (96), Rosenberg and Soskin (283), Boros (30), Cohn (49), Klotz (154), Schwimmer et al (298), Hoagland and Shank (119). This summary of the literature is not necessarily complete.

VITAMIN AND HORMONE METABOLISM

Vitamin A. Although the metabolism of almost every vitamin is closely associated with liver function, only the vitamin A plasma level has been utilized as a hepatic test (6,256). In acute hepatic disease and in infections such as pneumonia, the plasma vitamin A level drops, due largely to impaired release from the liver rather than to impaired intestinal absorption The drop of the plasma level in a few days far exceeds that occurring in healthy individuals placed on a vitamin A deficient diet for as long as a month Hepatic vitamin A stores are not necessarily diminished The deficient release of vitamin A from the liver has been explained morphologically by a displacement of vitamin A from its normal sites within the cell (as seen by fluorescence microscopy) (263) and chemically by a

Esterase. Atoxyl-resistant lipase is supposedly of pancreatic origin and not characteristically changed in liver disease. The rare instances of slight elevation have been attributed to secondary pancreatic involvement (133). The enzyme in serum which splits various esters, among them acetylcholine, is a protein formed only by the liver and thus is influenced by the same factors that regulate albumin, fibrinogen, and prothrombin formation. Serum esterase is reduced in severe liver disease such as advanced cirrhosis (93).

TABLE VI

Serum Alkaline Phosphatase Test: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results	
		Mildly elevated	Markedly elevated
Control patients	164	--	-
Acute infectious hepatitis	476	55 8	10.1
Toxic hepatitis	111	51.3	20 7
Cirrhosis	290	36 9	12 4
Extrahepatic biliary obstruction	434	28 4	65 0
Gallbladder disease	38	36 9	5 3
Tumor metastases	65	49 2	36 9
Chronic passive congestion	72	36 1	2 8
Miscellaneous	103	28 2	11 6
Total cases	*1753*		

* Herbert (114), Rothman et al (285), Giordano et al (90), Hanger (106), Gutman et al. (104), Klotz (154), Maclagan (183), Schwimmer et al. (298), van der Meer (351), Sherlock (306). This summary of the literature is not necessarily complete

Its faulty regeneration in liver disease after the specific inactivation of plasma esterase with diisopropyl fluorophosphate (DFP) offers a measure of the ability of the liver to synthesize the enzyme in particular and proteins in general (164) However, the complexity of the older method limits its value in the differential diagnosis of liver disease while its results equal that of simpler tests Recently a simpler promising method was described (219a, 357). In general, low values speak against biliary obstruction, provided metastases or cholangitis can be excluded, whereas higher values practically exclude cirrhosis (294)

and secrete it into the bile capillaries (84) In liver damage, either this formation is excessive (272) or (as better reconciled with the drop of serum phosphatase in severe hepatic insufficiency) the hepatogenic enzyme leaks out into the blood stream. The histologic distribution of phosphatase in the liver is in keeping with either hypothesis. Intravenously injected phosphatase, obtained by using the serum of dogs with ligated common ducts, is not rapidly cleared from the blood by the liver of a normal dog (40,84). However, serum phosphatase is inactivated by cyanide in contrast to liver phosphatase which is cyanide resistant (103). Further studies are required to clarify this problem.

Since the phosphatase is measured by its activity in units, the values vary with the amount and nature of the substrate used and the time of incubation in the several methods available (215). At present, the methods of Bodansky (26) and of King and Armstrong (144) and recently of Andersch *et al.* (10) are the most favored In adults, values above 4 Bodansky units and above 10 to 12 King-Armstrong units are abnormal; in children above 13 Bodansky units (26). For diagnostic use, it seems preferable to differentiate between mild and marked elevation The majority of cases of hepatitis and cirrhosis have a mild elevation, and only a few show a marked increase (Table VI) Occasionally, a marked elevation may be found in cirrhosis, even without jaundice In extrahepatic biliary obstruction this relationship is reversed. Hepatic tumor metastases may result equally in marked or mild elevations, whereas in chronic passive congestion only mildly elevated phosphatase is found in one-third of the cases It appears that a moderate increase (e g, 4 to 10 Bodansky units) is indicative of either hepatic disorder or milder degrees of extrahepatic obstruction, and excludes complete extrahepatic biliary obstruction. Marked elevation (e g, above 10 or 15 Bodansky units) suggests marked interference with the bile flow as seen typically in extrahepatic biliary obstruction or primary hepatitis with intrahepatic obstruction Diagnostically, the absence of marked increase of phosphatase has more significance than its presence.

Blood Diastase Reduction of the blood diastase occurs in hepatic disease and in biliary disease with secondary involvement of the liver (99). Elevation is associated with pancreatic disease.

Both are controlled by the liver. The reduced hippuric acid excretion in liver disease may be related to both, since some patients fail to show an increase in hippuric acid formation as a result of glycine administration(264). The functional state of the kidney influences both conjugation and excretion, and a normal nonprotein nitrogen or urea clearance should be required for the use of the test (156) In spite of this, the test has proved to be very helpful in the diagnosis of liver disease Two modifications are in use With the oral test, using 6 Gm. of sodium benzoate, healthy individuals excrete 3 or more grams of benzoic acid in the form of hippuric acid within 4 hours (265) The intravenous test, using 1 77 Gm. of sodium benzoate, is more sensitive, since faulty intestinal absorption is avoided and the response of the liver is tested in a shorter time, thus depriving it of the chance to compensate for a deficiency (267), normal excretion exceeds 0.70 Gm. of hippuric acid In both tests, excretion of hippuric acid in excess of the amount administered may occur due either to overstimulation of the liver (283), or, more likely to excretion of nonconjugated sodium benzoate, or to a technical error such as precipitation of sodium chloride in addition to hippuric acid (225) The results of the test are related to body size (299) and probably also to diuresis (181,289)

The test almost uniformly yields abnormal results in conditions with liver damage (Table VII), and is valuable in establishing its degree Since hepatic damage produced by biliary obstruction is detected early by this test (usually after 2 weeks), its value in the differential diagnosis between medical and surgical jaundice is limited to the earliest period and is not apparent from a summation of published data (Table VII) The test is valuable, however, in following the course of either medical or surgical jaundice It yields abnormal results in a high percentage of patients with tumor metastases to the liver and in a moderate number of cases with passive congestion

Benzoyl Glucuronate Excretion After ingestion of a test dose of benzoic *acid*, normal persons excrete all of it as hippuric acid, in contrast to *sodium* benzoate which may be excreted partly as benzoyl glucuronate, in addition to hippuric acid (315). In liver disease, however, the conjugation of benzoic acid with glycine resulting in hippuric acid is incomplete and part of it appears in the

Summary. The moderate increase of serum alkaline phosphatase in hepatic damage and the marked elevation in extrahepatic biliary obstruction render the determination useful, especially if combined with other tests. Serum esterase determinations appear promising

WATER METABOLISM

Oliguria (apparently related to sodium retention) is common during acute liver disease, while the start of convalescence is ushered in by an excessive diuresis; the observation is helpful in the prognosis of liver disease (135). During the acute phase of infectious hepatitis, plasma volume and interstitial body fluid space are increased, while plasma and urinary chlorides are decreased (165) A defect in neutralization of the antidiuretic principle of the pituitary by the damaged liver, rather than an inability of the damaged liver to hold water, is said to account for these changes. Following the intake of 1,500 cc. of water (water tolerance test), the urinary excretion in liver disease is less than normal and its peak is delayed (2) As this response occurs in primary hepatitis and secondary liver cell damage due to prolonged obstruction and is absent in noncomplicated biliary tract diseases, diagnostic significance has been ascribed to the water tolerance test (4) Its value is limited by many interfering factors such as edema, ascites, dehydration, and renal damage (165)

TRANSFORMATION TESTS

Since the liver transforms a large number of exogenous substances in one way or another, the response of the organism to the administration of almost any exogenous substance can be utilized as a hepatic test The number of hepatic tests can thus be multiplied at will The liver may transform a compound into another one by oxidation or, more commonly, by conjugation with various physiologic substances This transformation, often considered a detoxification, is usually a device to facilitate urinary excretion of the substance

Hippuric Acid Test. Sodium benzoate is conjugated with available glycine in the liver and probably also in the kidney (312) to form hippuric acid, which is, in turn, excreted in the urine. If a large amount of sodium benzoate is given, the amount of hippuric acid excreted will depend on two functions of the body: (*1*) supply of glycine, and (*2*) its conjugation with the benzoate (265).

Summary. Hippuric acid synthesis is valuable for the assessment of the degree of liver damage and for the follow-up of liver disease but has only limited value in the differential diagnosis of jaundice unless used early in the disease The glucuronate excretion test is simpler; it also becomes abnormal after prolonged extrahepatic biliary obstruction

DYE EXCRETION TESTS

Dyes can be removed from the blood stream in various ways, depending on their chemical structure, electropotential, and molecular size Most of them are excreted in the bile and urine The amount excreted through either route depends upon the chemical formula of the dye (131). In liver damage, dyes excreted normally by the bile appear in the urine in increased amounts. Impaired liver function thus may be indicated by retention of the dye in the blood, decreased excretion in the bile, or increased excretion in the urine. Originally it had been assumed that dyes were first taken up by the Kupffer cells and transmitted to the liver cells Recent observations indicate that bromsulfalein (213) and rose bengal (212) are immediately taken up by liver cells. Also histologic clearance of dye from the liver parenchyma has been considered a test of hepatic function (382).

Bromsulfalein Retention Sodium phenoltetrabromophthalein sulfonate (bromsulfalein) is excreted in the bile after its uptake by the liver This excretion depends on three factors · (*1*) the excretory ability of the liver cells, (*2*) the scavenger ability of the liver (and/or Kupffer) cells, and (*3*) the circulatory efficiency in transporting the dye to the liver (31,41,221) The influence of absorption and excretion by the liver cells can be separated by the simultaneous determination of the dye in blood and bile (41) The study of the biliary dye concentration by duodenal intubation may reveal liver cell impairment in the presence of intact blood clearance, particularly in incipient biliary obstruction or mild hepatitis Destruction of the dye in the tissues explains normal clearance, in the presence of complete obstruction with insignificant urinary dye excretion *Decreased clearance indicates either disturbance of hepatic circulation* (more important here than in any other hepatic test) or saturation of the liver with the dye due to impaired hepato-cellular function (213) There are variations in the amount of bromsulfalein

urine as benzoyl glucuronate. Any excretion of benzoyl glucuronate after benzoic acid ingestion is abnormal, and delayed excretion indicates severe liver damage (316). A carbohydrate meal or glucose infusion prior to the test may facilitate the preferential hippuric acid synthesis and thus produce normal results in the presence of abnormal liver function. Published reports, largely confirmed by our own observations, indicate that the results are similar to those of the hippuric acid test but that the technical aspects are far simpler than hippuric acid determinations

TABLE VII

Hippuric Acid Synthesis Test: Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Control patients	160	1 9
Acute infectious hepatitis	167	70 7
Recovering from hepatitis	24	20 8
Toxic hepatitis	31	80 6
Cirrhosis	319	84 5
Extrahepatic biliary obstruction	110	81 8
Gallbladder disease	153	34 0
Tumor metastases	93	92 5
Chronic passive congestion	24	52 5
Miscellaneous	315	68 2
Total cases	*1413*	

* Quick (266), Snell and Plunkett (317), Kohlstaedt and Helmer (156), Bartels (15), Rosenberg and Soskin (283), White et al (379–380), Rennie (275), Cohn (49), Machella et al (181), Mateer et al (206), Wade and Richman (358), Sherlock (306) This summary of the literature is not necessarily complete

Other Transformation Tests The following have been utilized as hepatic tests the excretion of oxycinchophen after administration of cinchophen (172), the excretion of oxysantonin in bile and urine after administration of santonin (76); the excretion of sulfuric acid compounds after administration of indole, *p*-cresol, phenol; the glucuronic acid excretion after administration of camphor, menthol, and sodium salicylate (173) They have all been discarded.

tion of the liver cells. Obviously, the results are influenced by Kupffer cell activity. Absence or delayed appearance of the dye in the bile signifies hepatic damage (173). The test is of no value in the absence of bilirubin excretion. However, it may indicate persistent liver damage in cholelithiasis if the appearance of the dye is delayed after restoration of biliary excretion. At present, the test is mainly used as a supplement to duodenal intubation

Other dye excretion tests, such as the use of phenoltetrachlorphthalein and phenolphthalein have only historical interest in that

TABLE VIII

Bromsulfalein Retention Test Number of Cases and Percentage of Abnormal Results as Reported by Various Workers*

Diagnosis	Number of cases	Per cent abnormal results
Control patients	145	1.4
Acute infectious hepatitis	317	82 8
Toxic hepatitis	29	82 7
Cirrhosis	368	87 2
Extrahepatic biliary obstruction	75	78 7
Gallbladder disease	536	42 7
Tumor metastases	69	92.7
Chronic passive congestion	73	61 6
Miscellaneous	360	62 5
Total cases	*1987*	

* Rosenthal and White (284), Schiff and Senior (293), Cantarow (38–39), Tu[illegible]
et [illegible]
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the roentgen-ray visualization of the gallbladder developed from them Nonvisualization of the gallbladder results not only from obstruction of the cystic or common duct, insufficient concentration of dye due to ill-functioning gallbladder mucosa, premature emptying of the gallbladder (as a technical error), or poor intestinal absorption, but also from inability of the liver to excrete the dye (tetraiodophenolphthalein). In this respect, cholecystography is a hepatic test This may also explain nonvisualization of the gallbladder in

given and the time intervals at which determinations are made The use of 5 mg. per kilogram of the dye, with readings after 45 minutes, seems to be preferable to 2 mg. per kilogram with readings after 20 minutes (205) because the increased burden placed on the liver permits better testing of its capacity (242). A reading after 30 minutes is preferred by some because of greater sensitivity (113). Results are not recorded in milligrams per hundred cubic centimeters, which would be more logical, but as per cent retention For this calculation, the plasma volume is arbitrarily considered as 50 cc. per kilogram of body weight. Normally, 0 to 6 per cent retention is found. Spectrophotometric readings eliminate interference by blood and bile pigments and are more accurate. Various clearance coefficients have been described (167,213).

The test shows only rare "false positive" reactions in the control group (Table VIII). It is very sensitive for the recognition of liver cell damage and is the method of choice in screening for hepatitis in preicteric phases and nonicteric types. It is of less value, though used, for recognition of sustained activity in the posticteric phase. It is used to detect cirrhosis and hepatic dysfunction in cholelithiasis without jaundice Abnormal results are very frequently found in carcinoma metastases (250). However, results may also be abnormal in infectious diseases without obvious liver damage and in chronic passive congestion In the presence of jaundice, the value of the test decreases with increasing bilirubinemia, and is therefore, as a rule, not recommended However, marked retention in incomplete biliary obstruction with only slight jaundice points to secondary hepatic damage. On the other hand, nearly normal clearance in the presence of jaundice indicates a common duct stone Allergic reactions occur following the use of serial tests

Rose Bengal Test. Blood clearance of rose bengal has been widely replaced by the bromsulfalein test. Jaundice and hemolysis do not interfere with its use (130). Nevertheless, multiple venipunctures, the short intervals between the drawing of samples so that the test is more susceptible to errors, and the danger of photosensitization in the patient are disadvantages Interpretations are identical to those of the bromsulfalein test (173)

Azorubin S Test. The excretion of intravenously administered azorubin S in the bile is measured This tests the excretory func-

tion of the liver cells. Obviously, the results are influenced by Kupffer cell activity. Absence or delayed appearance of the dye in the bile signifies hepatic damage (173) The test is of no value in the absence of bilirubin excretion. However, it may indicate persistent liver damage in cholelithiasis if the appearance of the dye is delayed after restoration of biliary excretion At present, the test is mainly used as a supplement to duodenal intubation.

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conditions with possible hepatic damage such as thyrotoxicosis, myxedema, and diabetes mellitus

Summary. The bromsulfalein test is particularly valuable in the nonjaundiced patient, and when used as a load test (5 mg./kg.) it may indicate the extent of hepatic damage. This and cholecystography have little value in the differential diagnosis of jaundice

HEMATOLOGIC ALTERATIONS

The liver influences the formation as well as the fate of the corpuscular elements of the blood. Therefore, hematologic alterations can, with proper precautions, be indicators of deranged hepatic function.

The erythrocyte maturation factor is stored in the liver. Despite normal formation of the maturation factor in the stomach, a picture resembling pernicious anemia may result from inability of the damaged liver to store it (385). However, assays for the maturation factor have not consistently revealed lowered amounts, even in severely damaged livers (292). Moreover, a megaloblastic marrow with a picture of pernicious anemia rarely occurs in liver damage; when it does, it is primarily in younger patients with cirrhosis and superimposed acute parenchymal damage (297). Macrocytosis with hypochromasia but not necessarily with characteristic bone marrow changes, on the other hand, is very common (19,384) and occurs rapidly after the onset of liver damage The red cells look like target cells. Since the enlargement of the cells compensates for the reduced hemoglobin concentration, and flattening for the increased diameter, the color index and volume index are about 1. The rapid appearance of these changes speaks against an influence upon the formation of erythrocytes but rather for an effect upon the circulating red cells (297). The macrocytic, hypochromic, flattened red cells have, for purely physical reasons, less tendency to swell. This explains their decreased fragility (or increased resistance) to hypotonic saline in acute or chronic liver damage (27). A moderate anemia (3–3.5 million red blood cells per cubic mm) is characteristic for cirrhosis without bleeding

Since, in cirrhosis, splenomegaly occurs primarily as a result of the portal hypertension, a hypersplenic effect may be detected in some instances It may influence the red cell count and be responsi-

ble for the anemia in Banti's syndrome, or may make itself felt on the formation of leukocytes Leukopenia is typical in the presence of acute, as well as of chronic, liver damage, and may have some diagnostic importance A dampened response to stimuli provoking leukocytosis is also seen Leukocytosis has diagnostic significance in the recognition of a bacterial infection of the portal triads in an extrahepatic biliary obstruction (purulent hepatitis (255)). Leukopenia primarily involves the granulocytes, resulting in a relative but rarely absolute lymphocytosis. The lymphocytes in infectious hepatitis assume atypical shapes somewhat similar to those in infectious mononucleosis

Thrombocytopenia may occur in hepatic insufficiency (381), and disappears with improvement It has been explained by a hypersplenic effect as well as by a specific hepatic dysfunction

Summary Hematologic observations provide multiple diagnostic clues in liver disease but cannot be used as clear-cut tests of hepatic function

HEPATIC BLOOD FLOW

Many hepatic tests measure the response of the liver to substances brought to it via the blood Results of these tests, especially the bromsulfalein clearance test, will thus depend on the efficiency of hepatic circulation and hepatic blood flow It is difficult to differentiate primary disturbances of hepatic function from those due to disturbed circulation By means of a recently devised ingenious technic based on determinations of hepatic blood obtained by venous catheterization, the hepatic blood flow in cirrhosis was found to be normal or only slightly lowered (32) Further development of these technically complex methods will enable better evaluation of the hepatic tests.

DUODENAL DRAINAGE

Three types of bile may be aspirated by duodenal intubation. "A" bile comes from the common and cystic ducts and contains some freshly secreted liver bile, the dark "B" bile represents gallbladder contents, and the light, golden-brown "C" bile is again the direct secretion from the liver

Inspection of the aspirated fluid may reveal absence of bile pigment, the most reliable means of establishing the diagnosis of biliary obstruction Failure to obtain "B" bile indicates either obstruction

conditions with possible hepatic damage such as thyrotoxicosis, myxedema, and diabetes mellitus.

Summary. The bromsulfalein test is particularly valuable in the nonjaundiced patient, and when used as a load test (5 mg/kg) it may indicate the extent of hepatic damage. This and cholecystography have little value in the differential diagnosis of jaundice.

HEMATOLOGIC ALTERATIONS

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Since, in cirrhosis, splenomegaly occurs primarily as a result of the portal hypertension, a hypersplenic effect may be detected in some instances. It may influence the red cell count and be responsi-

tion, may indicate impaired activity of the liver cells A further group, such as the serum globulin determination and the flocculation tests, reflects distorted activity of the liver as a whole. Hyperbilirubinemia and bilirubinuria, rise of serum alkaline phosphatase and of total cholesterol result from disturbed bile excretion

Reserve Hepatic reserve is best recognized by tolerance tests with physiologic substances The greater the load, the better this reserve will be evaluated. The galactose tolerance and bilirubin tolerance tests are good examples

Capacity Capacity tests which measure activity plus reserve deal primarily with exogenous substances Again, the measurement is more reliable the larger the load, a factor which has been stressed in selecting the amount of bromsulfalein to be used. The various dye excretion tests (205) and hippuric acid synthesis belong in this group.

As a whole, this classification has of more academic than practical value

Sensitivity Evaluation

The question of sensitivity of the various function tests, especially in comparison with each other, has been widely discussed. However, the problem does not lend itself to clear-cut answers Actually, it had been stated that no test known (in 1931) would indicate damage when less than 80 per cent of the liver was destroyed in the experimental animal (28) In liver disease, this factor is complicated by the tremendous regenerative ability of the liver, as seen in cirrhosis (130). The use of very sensitive hepatic tests is limited in some respects because minor alterations of liver function occur under physiologic circumstances, and almost every disease will lead to some nonspecific changes The more sensitive a test is made, the less specific it becomes Sensitive tests are used for establishment of the presence of liver damage, for screening purposes, and for the evaluation of the effect of drugs. They include the tests for prompt-reacting serum bilirubin, for urinary urobilinogen and bilirubin, some of the flocculation tests, especially colloidal gold, and bromsulfalein clearance The moderately sensitive tests which reflect definite but not yet severe liver damage, and the nonsensitive tests which record only severe liver damage, are more useful in the differential diagnosis of jaundice and in the management of hepatic disease

of the cystic duct or poor concentration by the gallbladder. Leukocytes and epithelial debris found exclusively in "B" bile point to an empyema of the gallbladder; and in "C" bile, to a cholangitis Cholesterol or calcium bilirubin crystals in the bile are found in cholelithiasis or choledocholithiasis (25). Bacteriologic examination may reveal infections of the gallbladder or biliary tract The difference in the icteric index between "B" and "C" bile measures the concentrating ability of the gallbladder. Urobilinogen, found in normal bile only in small amounts, may be markedly increased as the result of bacterial infection in the bile ducts (286), or possibly due to inability of the liver to reconvert it to bilirubin (76). The determination of bile acids in the bile has been considered as one of the most reliable methods of studying liver function, since bile acids are known to be a specific secretory product of the liver (76). However, no satisfactory method is available for their routine determination In carcinoma of the pancreas or pancreatolithiasis, tryptic enzymes may be absent from the aspirated fluid (25) The presence of gross blood indicates an ulcerating carcinoma of the ampulla

The disadvantages of duodenal drainage are the technical difficulties, the inconvenience to the patient, and the variable error due to the mixture of duodenal and biliary secretions.

Summary. Duodenal drainage offers information as to the functional state of the liver and biliary tract. However, the recent emphasis on roentgenographic examination of the gallbladder and the simpler laboratory tests have discouraged its widespread use

Dynamic Evaluation

A dynamic evaluation of the hepatic tests, though desirable, is rather difficult because the physiologic bases of most of the tests are vague and their results reflect multiple processes within the liver. In addition, some tests, such as those for urinary urobilinogen values, depend on the patency of the excretory passages as well as on the function of the liver cell.

Activity The blood albumin and glucose levels, the plasma prothrombin response to vitamin K, and the cholesterol–cholesterol ester ratio mirror the activity of the liver cells. Other tests, such as those *pertaining to amino-aciduria and benzoyl* glucuronate excre-

influenced by the diet. The intravenous administration of glucose may render the hippuric acid synthesis and the glucuronate excretion test normal even though the liver is damaged (316).

Pregnancy Changes in hepatic function have been observed during normal pregnancy. These usually appear near term and disappear by the eighth or ninth day postpartum (248). At term, 35 per cent of women have abnormal cephalin flocculation (358) and hippuric acid synthesis (248). In the various toxemias of pregnancy, the incidence of abnormal results is similar to those in normal pregnancies at term It is not known whether the changes in liver function are a cause or an effect of the toxemia, and their routine use in obstetrics is therefore not recommended (322). Hepatic blood flow is normal in pregnancy (229).

Menstruation. On the first day of the menstrual period there is a sharp drop in hepatic efficiency, as measured by the ability to synthesize hippuric acid (112).

Infancy and Childhood. Most of the tests can be adapted with some modification for pediatric use. The tolerance tests require allowance for the smaller size; the oral galactose tolerance test can be used in children when 0 5 Gm per kilogram body weight is administered Only the neonatal period needs special considerations. Icterus neonatorum, the etiology of which is subject to changing concepts (373), influences the pigment metabolism In rare instances, it may be associated with disturbances of the carbohydrate metabolism (394) The prothrombin of the infant is markedly reduced at birth if vitamin K has not been given to the mother (141). The prothrombin time, if determined with the Quick method, is prolonged on the second day, reflecting the peak of the hemorrhagic tendency The cephalin–cholesterol flocculation is often abnormal in the first weeks of life, as is the levulose tolerance or bromsulfalein dye excretion Bilirubin tolerance, however, does not appear altered (288). Serum alkaline phosphatase is high in normal children, and rises even higher in liver disease (272). Thymol turbidity is low in newborns (62).

Senility Abnormal results of hepatic tests are common in persons over 60 years of age without clinical evidence of hepatic disease. They are found in the following tests in order of decreasing incidence: cholesterol ester ratio, cephalin flocculation, bromsulfalein

Representative of the former group are the intravenous galactose tolerance test, serum albumin and globulin analysis with most of the flocculation tests, cholesterol–cholesterol ester ratio, intravenous hippuric acid synthesis, and glucuronate excretion The nonsensitive group includes the oral galactose tolerance and hippuric acid synthesis (206), blood sugar, prothrombin, urinary amino acid, and total cholesterol determinations (266). In the presence of equal degrees of jaundice (except in terminal stages), abnormal results with nonsensitive tests speak more for primary hepatic disease than for extrahepatic obstruction.

It has been stated that the bromsulfalein clearance, intravenous hippuric acid test, and cephalin flocculation reaction are of equal sensitivity (207) ; also that bromsulfalein and rose bengal clearance, as well as serum alkaline phosphatase, are comparable (69). However, such relations are not fixed, since in different stages of the same disease different tests reveal varying degrees of sensitivity. Moreover, the marked regenerative ability of the liver which masks the degenerative changes makes statements as to sensitivity questionable (130)

Evaluation of Physiologic Fluctuations

Diet. Deficient diets may produce liver damage, and abnormal results of hepatic tests have been found in animals with experimental cirrhosis, necrosis, or fatty changes due to dietary factors (117,277) There are scattered reports on the effect of frank malnutrition or vitamin deficiencies upon hepatic function in man Urinary urobilinogen may be elevated and fecal urobilinogen diminished, although the blood sugar, glucose tolerance, alkaline phosphatase, and hippuric acid synthesis (intravenous) are not necessarily altered (308). Repatriated prisoners of war revealed a variable incidence of abnormal bromsulfalein retention and cephalin flocculation (152,211)

The effect of physiologic fluctuations due to the preceeding diet on the results of the hepatic tests is of more practical interest here The diet may influence the galactose tolerance test because the glycogen and the fat stores of the liver determine glycogen formation and deposition The hippuric acid test obviously depends on the glycine intake (15). The benzoyl glucuronate excretion test is also

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Respiratory excursions may cause lacerations; these are best avoided by rapid aspiration. A recently advocated medial approach to the left lobe reduces the chances of reaching larger vessels and the danger from respiratory movements, and facilitates surgical intervention in the cases of uncontrollable hemorrhage (89). Several types of needles have been recommended (59,89,128,261,345,352, 354) The lateral approach with the Vim-Silverman needle is at present the most widely used, rendering the best specimens

Aspiration is the simplest of the three methods of liver biopsy, which explains its recent wide application. However, hemoperitoneum as a result of laceration of the liver and hemothorax due to nicking of an intercostal artery have led to death in isolated instances. Aspiration biopsy, therefore, is the most dangerous of the hepatic tests Some authors have reported large series without fatalities (Table IX). Nevertheless, in the literature reviewed, a total mortality of 0 5 per cent is recorded. Precautions and contraindications are therefore of utmost importance. Although no complete agreement exists (305), biopsies should not be performed in the presence of hypoprothrombinemia (below 60%), severe cholemia, chronic passive congestion of the liver, systemic hypertension, and hemorrhagic diseases in general (261). It should only be performed on hospitalized patients who must be carefully watched for at least 12 hours after the biopsy, with frequent pulse and blood pressure determinations Blood transfusions should be administered at the slightest suggestion of hemorrhage, followed by laparotomy in the rare instances in which alarming symptoms appear

Comparison with necropsy specimens reveals that the tissue core usually obtained is large enough for recognition of diffuse hepatic disease. Tumors or other focal lesions which are not reached by the needle, and cirrhosis represent sources of error The small core may not be typical of the entire liver; in some instances, normal architecture is seen if the center of a large nodule in a cirrhotic liver is aspirated In other instances, scars or marked inflammatory changes in the periportal tissue aspirated may present an exaggerated picture. Obviously, in such instances a peritoneoscopic biopsy will give more accurate results. In view of the different histologic pictures in biopsy and necropsy material, special experience on the part of the pathologist in interpreting biopsy specimens is required

clearance, intravenous and oral hippuric acid synthesis (270). The impairment of the hippuric acid synthesis is apparently due to a defect in the supply of glycine rather than to an impairment of conjugation (331).

Evaluation with Reference to Morphologic and Functional Changes

A good correlation cannot be expected between hepatic tests and specific disease entities, since any disease usually consists of several histopathologic or physiopathologic phenomena. However, correlation of the tests with individual morphologic or functional phenomena, independent of the clinical diagnosis, is more promising Such correlations may serve two purposes. They may assist in appraising the value of a hepatic test and in clarifying the functional significance of an observed morphologic change. Several attempts have been made to correlate histopathologic findings with results of hepatic tests in experimental animals (69). However, the results were not consistent This correlation in man has been aided by the use of liver biopsies (82,122,147,261,306) Correlation with necropsy material is less feasible because of the marked effect of agonal and postmortem histologic alterations (253) and the usually considerable time interval between performance of hepatic test and death.

LIVER BIOPSY

There are three types of liver biopsy. (*1*) Excision at laparotomy, which usually provides subcapsular parenchyma. This may normally reveal fibrotic changes, and is not necessarily representative of the entire liver. Obviously, as a diagnostic method, surgical biopsy is limited (*2*) Forceps biopsy during peritoneoscopy (120,209) permits selection of material from visualized areas. It offers advantages in focal changes, such as tumor metastases, in correlation of the histologic picture with the gross appearance, in obtaining several pieces for use with different fixatives, and finally in the immediate recognition of hemorrhage However, the subcapsular origin of the piece obtained may obscure the histologic picture, just as with surgical biopsy. (*3*) In aspiration biopsy, a needle is inserted either into the anterior surface of an enlarged liver below the costal arch or through an intercostal space, chiefly in the right midaxillary line, usually through the pleura. A small core of tissue is aspirated

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Liver biopsy may be helpful in several respects. (*1*) It assists in the differential diagnosis of liver disease, especially in distinguishing medical from surgical jaundice. This requires the greatest experience, because the histopathology of the different diseases is still not too clearly described. As a rule, the distinction between medical

TABLE IX

Number of Cases Examined by Various Investigators and Number of Fatalities in Liver Biopsies*

Author	Route	No of biopsies	Number of fatalities
Olivet (243)	Anterior intercostal	140	3
Iversen, Roholm (128)	Lateral intercostal	160	0
Baron (14)	Anterior subcostal	49	1
Tripoli, Fader (345)	Anterior subcostal	14	0
Chiray *et al.* (46)	Lateral subcostal	41	0
Roholm *et al.* (280)	Lateral intercostal	297	2
Dibble *et al.* (64)	Lateral intercostal	61	1
Van Beek, Haex (349)	Lateral intercostal	200	0
Raby (269)	Lateral intercostal	—	2
Gilman, Gilman (89)	Subxiphoid into left lobe	506	1
Sherlock (305)	Lateral intercostal	264	2
Davis *et al* (59)	Anterior subcostal	68	0
Herrera, Pardo (115)	Anterior subcostal	72	0
Volwiler, Jones (354)	Anterior subcostal	278	1
Kumpe *et al.* (158) Safdi *et al.* 287)	Lateral intercostal	357	0
Giraud, Cazal (91)	Lateral subcostal	200+	0
Hoffbauer (120, 121)	Anterior subcostal, or Lateral intercostal	96	0
Koch, Karl (155)	Lateral subcostal	100	0
Harris (109)	Lateral intercostal	90+	0
Buck (37)	Anterior subcostal	56	1
Total		*3055*	*14*

* This summary of the literature is not necessarily complete.

and surgical jaundice or between intrahepatic and extrahepatic biliary obstruction becomes easier the older the process is. Diagnoses made by clinical and laboratory observations were improved by liver biopsy in 3.6 per cent of 192 cases (261). On the other hand, in 5.3 per cent of these cases, the biopsy diagnosis without knowledge of clinical and laboratory factors differed from the final diag-

nosis and was, therefore, most probably wrong. In another series of 170 cases, the results of liver biopsy changed an incorrect clinical diagnosis to a correct one in 24 cases (158); 8 biopsies failed to reveal subsequently demonstrated lesions (2) It permits diagnosis of hepatomegalies, including recognition of cirrhosis, amyloidosis, primary or metastatic carcinoma, lymphomatous, parasitic, or granulomatous diseases, especially of Boeck's sarcoid (or as recently reported, of miliary tuberculosis (53)). (3) It enables evaluations of therapy, such as the effect of lipotropic substances in cirrhosis (83, 153,355). (4) It provides general academic information concerning the histopathology of the various liver diseases, correlation of histologic and functional findings, etc. There are many recent reports on the use of aspiration biopsy in liver disease (47,58,344).

Although liver biopsy, especially by aspiration, is of great diagnostic value, so long as fatalities occur, its use appears justified only if after exhaustion of clinical and other laboratory procedures the diagnosis remains questionable.

CORRELATION WITH MORPHOLOGIC PHENOMENA

Recent investigations, some statistical (82), point to numerous correlations between hepatic tests and morphologic phenomena Some of these relations may be only incidental associations rather than causative in nature.

Generalized Liver Cell Damage. This is characterized by well-defined changes in the nuclear or cytoplasmic structure in the great majority of the epithelial liver cells Such damage shows the most significant statistical correlation with abnormal thymol turbidity and cephalin–cholesterol flocculation (82,262). This correlation is absent in biliary hepatitis, in which marked liver cell damage may be seen in the presence of normal flocculation tests Earlier in this paper doubt was expressed as to whether the flocculation tests indicate liver damage or associated mesenchymal involvement There is also very good correlation between liver cell damage and reduced albumin–globulin ratio This is also present in biliary hepatitis Reduction of albumin seems to be the responsible factor There is almost as good a correlation with bromsulfalein retention, whereas a fair one is found with reduced plasma vitamin A, prolonged prothrombin time, markedly raised serum bilirubin, and a moderate elevation of the alkaline phosphatase level (4–10 Bodansky units).

No correlation was found between liver cell damage and total protein concentration. No parallelism was found with total serum cholesterol, urinary and fecal urobilinogen, sedimentation rate, and elevation of the alkaline phosphatase above 10 Bodansky units Although no statistical correlations have been made, it is safe to assume that the galactose tolerance test, oral and intravenous hippuric acid tests, benzoyl glucuronate excretion, coproporphyrin I excretion, poor vitamin K response of the prothrombin time, low blood sugar, macrocytosis, and amino-aciduria are related to liver cell damage. In general, the number of hepatic tests yielding abnormal results increases with the severity of the disease (257). The tests with good correlations are largely those listed as indicating hepatocellular damage (72,257,370).

Focal Necrosis. Discrete lesions, such as focal necrosis or granulomas, show no statistical correlation with any of the tests (82). Patchy disease with more than 80 per cent involvement of the parenchyma will show results similar to a mild diffuse damage (245). Also, scattered inflammatory changes seen in persons without clinical liver disease (infiltrative hepatitis) do not correlate with results of hepatic tests (208).

Regenerative Changes These show a fairly good correlation with increased thymol turbidity (82). This is possibly explained by the usually associated proliferation of the Kupffer cells or periportal inflammatory infiltration, as seen in protracted infectious hepatitis.

Distorted Reconstruction of Lobular Pattern. This phenomenon which is characteristic for cirrhosis was correlated with cephalin flocculation, increased thymol turbidity, and elevated sedimentation rate (82) Probably all three are related to the usually concommittant mesenchymal reaction

Periportal Inflammatory Activity. In the available statistical comparison, only an increased sedimentation rate revealed any correlation with periportal activity (82)

Fatty Metamorphosis of the Liver Cells No correlation with this and any of the tests just mentioned was found (82). However, in dogs, bromsulfalein and rose bengal retention was noted when the fat content of the liver exceeded 25 per cent (130).

Hyperactivity of the Kupffer Cells This was found to be well

correlated with reduced albumin–globulin ratio and marked hyperbilirubinemia (82). The correlation with the former is apparently due primarily to an elevated globulin. The one with the latter is explained by the presence of large, bile-laden Kupffer cells in severe jaundice. Recent personal observations suggest a close correlation of Kupffer cell activity to the flocculation tests.

CORRELATION WITH FUNCTIONAL PHENOMENA

Biliary Obstruction Biliary obstruction, in the sense of reduced bile flow or, chemically speaking, reduced bilirubin secretion into the duodenum, may be due to an extrahepatic lesion or may occur in intrahepatic processes such as acute hepatitis or cirrhosis The pathogenesis of the phenomenon in the former is obviously mechanical. In the latter, it is not fully established In some instances of cirrhosis, a cholestatic phase may be produced by constriction of the smaller bile ducts by an encircling fibrosis In other instances, regurgitation through the cholangioles without morphologic evidence of damage or obstruction has been assumed (369). Since the site of regurgitation of bile in the intrahepatic and extrahepatic form has been localized in the cholangioles, the tests indicating this form of jaundice have been grouped under the term cholangiolar (370) or hepatocanalicular (72). However, in view of the still unsolved mechanism of regurgitation, it may be better to list the following phenomena as indicating marked interference with the bile flow: reduction of urinary urobilinogen, marked reduction of fecal urobilinogen, elevation of serum alkaline phosphatase above 15 Bodansky units, and elevation of total serum cholesterol above 300 mg per hundred cubic centimeters Hyperbilirubinemia and bilirubinuria might be added to this group Whatever the pathogenesis of this phenomenon may be, functionally it represents a complete or incomplete biliary obstruction

Complete Biliary Obstruction This condition is most often extrahepatic (tumors, strictures, and stones, short lived in the last); rarely, it is intrahepatic and then usually only of short duration (326). It is characterized by complete absence of bile pigment from the duodenal aspiration, of urobilinogen from the urine, and absence or marked reduction of fecal urobilinogen.

Incomplete Biliary Obstruction Its extrahepatic form is especially common in choledocholithiasis where the stone may produce

a ball valve effect. Results of the hepatic tests are the same as in complete obstruction, although they usually are less abnormal. Alkaline phosphatase, for instance, may be below 10 Bodansky units Urinary urobilinogen is an exception, since in choledocholithiasis fluctuating excretion curves are seen (324, 365).

Hemolysis. This is indicated by a moderate elevation of the total serum bilirubin, with hardly any elevation of the prompt-reacting fraction, lack of urinary excretion of bilirubin and bile acids, and a high fecal and occasionally high urinary urobilinogen content in the presence of jaundice In addition, there is spherocytosis retic- locytosis, hyperchromasia with [illegible]

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Evaluation with Respect to Diseases

Hepatic tests, with the possible exception of liver biopsy, do not permit the diagnosis of any hepatic disease because of the multiplicity of derangements present However, under a given circumstance, one test may become characteristic of a clinical entity and may thus permit its specific diagnosis.

Viral (Infectious) Hepatitis Epidemiologic experiences during World War II and experiments with human volunteers support the hypothesis that a considerable number of the conditions previously called catarrhal jaundice in the benign form and acute yellow atrophy in the fatal form are due to a virus (193) The differentiation between hepatitis transmitted by oral routes and homologous serum jaundice transmitted by parenteral routes cannot, as yet, be made by laboratory tests, although the latter form is generally more severe (233) The results of hepatic tests in the different stages of viral hepatitis (110,119) have been established by well-controlled studies on inoculated human volunteers (234) In the preicteric stage, bromsulfalein retention and bilirubinuria are present, while the total blood bilirubin is not much elevated (110,234) The elevation is largely due to increase in the prompt fraction Increased urinary urobilinogen and abnormal cephalin flocculation and thymol turbidity appear a little later in the preicteric phase (110,234), whereas reduction of albumin becomes apparent after the appearance of jaundice (110) In the fully developed stage, tests indicative of liver cell damage show aberrant results, depend-

ing upon the severity of the disease. The results of flocculation tests are uniformly abnormal (Tables II–IV). In some patients, the tests for marked interference with the bile flow show abnormalities. Complete biliary "obstruction" is found more often in homologous serum jaundice than in the other variety (327). In the subsiding stage, bilirubinuria disappears before hyperbilirubinemia, and thereafter the flocculation tests, bromsulfalein retention, and the elevated sedimentation rate return to normal (110). The last to disappear is the elevated thymol turbidity (163). In later stages, this elevation is primarily due to changes in the globulin fraction, whereas in earlier stages it is due to increased lipids in the serum (162). In addition to the increased thymol turbidity in instances with delayed healing, bromsulfalein retention in the blood, delayed biliary excretion of bromsulfalein, abnormal bilirubin tolerance, and other abnormalities are recorded (150,386). This may occasionally represent a transition into chronic hepatitis (237,310) and possibly into cirrhosis, especially if associated with an elevated sedimentation rate, increased urinary urobilinogen excretion, and hyperbilirubinemia One sequel is cholangiolitic cirrhosis characterized by an almost complete absence of abnormal results with tests indicating liver cell damage, despite evidence of marked interference with bile flow (369). A grave prognosis is indicated by highly abnormal results of the tests indicating liver cell damage, especially amino-aciduria (leucine and tyrosine), a marked drop of total cholesterol and cholesterol ester ratio (245), or an abnormal Takata-Ara test (8). Nonicteric hepatitis is suggested by bromsulfalein retention, cephalin flocculation, increased thymol turbidity, and elevated urinary excretion of urobilinogen (13,234) Azotemia is rare in any of the forms.

Histologically, the biopsy findings are rather characteristic (193), and may permit differentiation from other forms of hepatitis (255) There is diffuse, somewhat centrally accentuated liver cell damage with uniform hyalinization of the cytoplasm of isolated cells (Councilman bodies), marked mesenchymal reaction indicated by proliferation of the Kupffer cells, and/or infiltration of the intralobular parenchyma and the portal triads with mononuclear cells (64,193) The last may be the only persisting lesion in the protracted form (193) Transition into cirrhosis has also been demonstrated (310).

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Hemolysis. This is indicated by a moderate elevation of the total serum bilirubin, with hardly any elevation of the prompt-reacting fraction, lack of urinary excretion of bilirubin and bile acids, and a high fecal and occasionally high urinary urobilinogen content in the presence of jaundice In addition, there is spherocytosis, reticulocytosis, hyperchromasia with microcytosis, a high degree of regenerative activity of the bone marrow, and increased red cell fragility (56)

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lein retention (180), elevated urobilinogen and blood tyrosine (54) were reported. In diffuse amebic hepatitis, elevation of the urinary urobilinogen, decreased total serum protein, abnormal hippuric acid synthesis, reduced galactose (319) or levulose tolerance (100), bromsulfalein retention (36), and abnormal cephalin flocculation (311) have been reported. The liver cell damage in hyperthyroidism may belong to this group; 45 to 90 per cent of all cases show some functional hepatic impairment as measured by galactose tolerance (9,188), hippuric acid synthesis (15), bromsulfalein clearance, and prothrombin time (210). Hepatic tests are considered of diagnostic value; they may be superior to the determination of the basal metabolic rate in the follow-up of the disease (295).

Biliary Hepatitis. Uncomplicated extrahepatic biliary obstruction results in biliary hepatitis, probably due to retention of bile acids It therefore depends on the degree and duration of the obstruction; thus, it is common in the malignant and complete type of biliary obstruction, but also occurs, although later, in incomplete or benign obstruction (219,255). This represents a variety of toxic hepatitis, and the distinction from it by functional and histologic laboratory findings is sometimes difficult. Some degree of liver damage, which is recognizable by a drop in the cholesterol ester ratio, reduction in serum albumin, or bromsulfalein retention occurs after a week or two of complete obstruction. Results of other tests, such as galactose tolerance, hippuric acid synthesis, or benzoyl glucuronate excretion, become abnormal after a longer period of obstruction, after about 2 weeks for the intravenous tests and more than 4 weeks for the oral tests including glucuronate excretion Gradually, with progressing obstruction and approaching cholemia, all tests for hepatic damage show abnormal results and azotemia appears (245) The flocculation tests are exceptions. They are, as a rule, normal or slightly abnormal (thymol turbidity below 7 units) despite severe liver cell damage. This peculiarity may be due to lack of increase in gamma globulin concentration (reflected by a low zinc sulfate turbidity (259), which in turn is associated with a lack of mesenchymal reaction; in addition some flocculations are depressed by regurgitated biliary substances (255a) [illegible] inogen is absent from the urine in complete obstruct[illegible]

Infectious mononucleosis is associated with jaundice in about 10% of the cases. In such instances, differentiation from viral hepatitis may be difficult. Even without jaundice some hepatic tests, such as cephalin flocculation, thymol turbidity, colloidal gold, bromsulfalein clearance, and serum alkaline phosphatase tests may give abnormal results (35,42,50,78,86).

Toxic Hepatitis. Recognized chemical, pharmaceutic, or endogenous poisons may cause toxic hepatitis (246). However, the term is also tentatively applied to cases with a similar clinical and histologic picture in which, however, the offender cannot be demonstrated (255). In the fully developed form, results of all tests for liver cell damage may be abnormal, the degree depending on the severity of the disease. One characteristic difference from viral hepatitis is that results of the flocculation tests are normal or only slightly abnormal in about 35 per cent of the cases and that the serum nonprotein nitrogen is often elevated due to associated renal damage (255). Marked interference with bile flow is more commonly found in the toxic than in the viral hepatitis (with the possible exception of homologous serum jaundice (326)) In some forms, as arsenical hepatitis, little evidence for liver cell damage can be detected and the signs of marked interference with the bile flow predominate (107) Chronic alcoholism commonly produces abnormal results in hepatic tests, especially in the determination of bromsulfalein retention, serum bilirubin and urinary urobilinogen (353) Ethyl alcohol in small doses reduces only the galactose tolerance (101)

Histologically, marked liver cell damage is seen In some instances, fatty metamorphosis is conspicuous in characteristic contrast to the viral form There is spotty coagulation necrosis of the cytoplasm indicating slow cell death The mesenchymal reaction, as a rule, is not impressive, if it is present, segmented leukocytes may predominate.

In specific varieties of toxic hepatitis, abnormal results of the hepatic tests are recorded In Weil's disease (186,235) or yellow fever, abnormal cephalin flocculation, elevated thymol turbidity, abnormal colloidal gold reaction, and elevated serum bilirubin have been described In malaria, abnormal cephalin flocculation (116), abnormal colloidal gold and thymol turbidity tests (42), bromsulfa-

lein retention (180), elevated urobilinogen and blood tyrosine (54) were reported. In diffuse amebic hepatitis, elevation of the urinary urobilinogen, decreased total serum protein, abnormal hippuric acid synthesis, reduced galactose (319) or levulose tolerance (100), bromsulfalein retention (36), and abnormal cephalin flocculation (311) have been reported. The liver cell damage in hyperthyroidism may belong to this group; 45 to 90 per cent of all cases show some functional hepatic impairment as measured by galactose tolerance (9,188), hippuric acid synthesis (15), bromsulfalein clearance, and prothrombin time (210). Hepatic tests are considered of diagnostic value; they may be superior to the determination of the basal metabolic rate in the follow-up of the disease (295).

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lein retention, elevated urinary urobilinogen, and abnormal results in hippuric acid synthesis, benzoyl glucoronate excretion, galactose tolerance and flocculation tests are usually found. Serum albumin is lowered, as a rule. The marked gamma globulin increase is the most characteristic sign (98,204,278) While these abnormal results in the various tests are rather uniformly seen in cirrhosis, they may also be found occasionally in chronic passive congestion and hepatic tumor metastases. The findings in liver biopsies are usually diagnostic, and may thus be superior to those of the other hepatic tests

In cirrhosis with jaundice, acute liver cell damage is superimposed upon or part of the original disease Most tests for liver cell damage show abnormal results; in the cholestatic phase, occurring in the Laennec as well as the postnecrotic form, the tests indicative of marked interference with the bile flow are also abnormal. Most hepatic tests do not permit the differentiation from acute hepatitis. Only those indicating high gamma globulin concentration, such as the Takata-Ara test or gamma globulin or zinc sulfate turbidity (above 25 units), may be helpful In addition, a markedly increased thymol turbidity in the face of only a slight increase in the zinc sulfate turbidity suggests acute hepatitis (328). Liver biopsy aids in this differentiation, although the correlation between the degree of functional impairment and morphologic manifestations requires further study (355). Biliary cirrhosis (as a result of noninfected, prolonged, extrahepatic biliary obstruction) deserves special mention because the flocculation tests may occasionally not reveal abnormalities despite severe liver damage. Cholangiolitic cirrhosis was referred to before

The differentiation of the various etiologic forms of cirrhosis by functional tests is very difficult, with the possible exception of biliary cirrhosis Biopsy examination rarely differentiates postnecrotic from Laennec's cirrhosis It may permit recognition of biliary cirrhosis and of the more common cholangitic variety due to bacterial infection associated with extrahepatic obstruction

Tumor Metastases to the Liver The hepatic tests are quite often abnormal in the presence of hepatic metastases, as shown by increased thymol turbidity, decreased cholesterol esters, and abnormal cephalin flocculation, galactose tolerance, and colloidal gold tests in about 50 per cent of the cases (Tables I–VIII) The recorded dif-

due to liver damage in the incomplete form Tests indicating marked *interference with bile flow show abnormal results*

In biopsy specimens (255), evidence of bile stasis exceeds that of liver cell damage. Most of the bile casts are found in the centers of the lobules The portal triads show an encircling fibrosis in later stages, which may progress to biliary cirrhosis. The severe form reveals central necrosis with a subdued mesenchymal reaction, but with proliferated Kupffer cells laden with bile pigment In protracted cases, biliary necrosis of the parenchyma and foreign body *reaction around bile extravasations from the larger bile ducts are* noted These latter changes are observed primarily in extrahepatic biliary obstruction.

Purulent Hepatitis. *Infection of the portal triads* may be due to bacteria which may enter through the bile ducts or via the portal tributaries (pylephlebitis). More commonly, they pass through the intralobular sinusoids and localize around the lymphatics of the portal triads (perilymphangitis) Simple perilymphangitis is seen *in any disease involving the abdominal viscera. However, abnormal* results in the hepatic tests are not necessarily present More severe inflammation of the portal triads (purulent hepatitis (255)) may complicate biliary hepatitis without any relation to the degree and duration of the obstruction It is more common in benign obstruc*tion with infection than in malignant obstruction It may* accentuate the liver cell damage and the flocculation tests may become abnormal (255). Thus, the results of the hepatic tests simulate those seen in primary medical hepatitis and only clinical evidence of septicemia may permit the recognition of a surgical condition.

Histologically, dense infiltration of the portal triads by segmented leukocytes is characteristic for purulent hepatitis However, correlation between the morphologic findings and the results of the function tests, especially flocculation tests, is not convincing

Cirrhosis Three problems arise in the diagnosis of cirrhosis *(1)* differentiation from other diseases, especially the *hepatomegalies*, in the absence of manifest jaundice, *(2)* distinction from other hepatic diseases, primarily from acute hepatitis, in the presence of manifest jaundice, *(3)* recognition of the various types of cirrhosis

In cirrhosis, in contrast to most other hepatomegalies, *bromsulfa-*

lein retention, elevated urinary urobilinogen, and abnormal results in hippuric acid synthesis, benzoyl glucoronate excretion, galactose tolerance and flocculation tests are usually found. Serum albumin is lowered, as a rule. The marked gamma globulin increase is the most characteristic sign (98,204,278). While these abnormal results in the various tests are rather uniformly seen in cirrhosis, they may also be found occasionally in chronic passive congestion and hepatic tumor metastases. The findings in liver biopsies are usually diagnostic, and may thus be superior to those of the other hepatic tests.

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ferences in the various tests are probably incidental. Hippuric acid synthesis and bromsulfalein retention seem to be uniformly abnormal. Serum alkaline phosphatase is often significantly elevated. Serum bilirubin determination is apparently of no value (250). Liver biopsy may reveal metastases if the biopsy needle happens to puncture the lesion.

Passive Congestion. Urinary urobilinogen is elevated and bromsulfalein retention is present before most of the other tests become abnormal but eventually any of the tests for liver cell damage may reveal aberrant results (45). In general, the tests show abnormal results in about half of the cases (Tables I–VIII). The colloidal gold test is abnormal and the alkaline phosphatase is slightly elevated in only a third of them. The relatively low incidence of abnormal results in the galactose tolerance test may be due either to the small number of cases considered or to impaired intestinal absorption. Histologically, it is characterized by central lobular necrosis, with blood stasis and dilatation of the central vein.

Gallbladder Diseases Conditions associated with jaundice due to some extrahepatic biliary obstruction are discussed under biliary hepatitis However, even in gallbladder disease without jaundice, impairment of liver function is often recorded. Urinary urobilinogen excretion is frequently elevated. The tests listed in Tables I to VIII reveal abnormal results in about one-third of the cases In biopsy specimens, inflammatory changes are often found, especially in the periportal fields; these show all gradations from a simple perilymphangitis to purulent hepatitis (208,217). Occasionally focal necrosis may also be seen The significance of these functional and morphologic changes is problematic.

Gastrointestinal Diseases. In the various gastrointestinal disorders, especially peptic ulcer, carcinoma of the stomach and colon, ulcerative colitis, and pancreatitis, abnormal results are reported by various investigators. The findings are erratic, with the exception of those in cancer of the gastrointestinal tract (1). (These cases were included under "Miscellaneous" in the tables, since an insufficient number of cases have usually been recorded) In biopsy specimens, similar findings were noted as in gallbladder diseases (208,217).

Hepatomegalies. The various hepatomegalies are easily recognized by biopsy Abnormal results in the hepatic tests are seen in

amyloidosis (343) (bromsulfalein retention, reduced serum albumin, diminished glucose tolerance, and macrocytosis) or protozoal diseases such as schistosomiasis (10 per cent of all tests abnormal (175)), amebic abscess of the liver (similar to amebic hepatitis discussed above), and kala-azar (abnormal cephalin flocculation and thymol turbidity tests (227)). Abnormal results may also be found in the various storage diseases and lymphomas with infiltration of the liver.

Practical Evaluation

The greatest challenge in the clinical use of the hepatic tests is their practical evaluation, which is the ultimate measure of their usefulness. This must be based on the knowledge gained from the other types of evaluations. The attempt to classify the tests for practical use is associated with the effort to develop systems of dovetailing the results of two or more hepatic tests with each other. Multiple hepatic tests are performed for several reasons. The tests may indicate different types of involvement, as, for instance, liver cell damage versus marked interference with bile flow. However, even for establishing the presence of liver damage, several tests are recommended since impairment of different functions does not appear simultaneously (dissociation of liver function (198)) In addition, no test shows abnormal results in all instances of hepatic disease ("false negative" reactions), and there is a variable number of abnormal results in the absence of demonstrable liver involvement ("false positive" reactions) By adding the results of several tests, the probability of correct diagnosis is increased. An outstanding system among the many devised (123,130,205,283,298,340) is the liver profile (122,370).

The answers to the questions listed below represent only one, surely controversial, attempt to group the hepatic tests, without claiming that it is necessarily the best or final one

(*1*) Which system of hepatic tests aids in the differentiation between medical and surgical jaundice?

This "therapeutic differential diagnosis" (129) is the most important field for hepatic tests. In principle, the results of tests indicative of liver cell damage are abnormal while those for marked interference with bile flow are normal in medical jaundice, and vice versa in surgical jaundice However, exceptions to this principle

are: (*a*) secondary liver cell damage in surgical jaundice due either to prolonged obstruction (biliary hepatitis) or to secondary bacterial infection of the portal triads (purulent hepatitis); (*b*) incomplete extrahepatic biliary obstruction, resulting in fluctuating results of tests indicative of marked interference with bile flow in

285 cases
MEDICAL
SURGICAL
151 L+B-
L-B+ 35
L+B+ 99
148
Fluctuating
urinary urobilinogen
Arsonical history
or cholangiolitic cirrhosis
Ceph floc
Mass
32
2
1
3
61 +
- 38
Mass metastasis, or history of
repeated attacks of biliary colic
Sepsis
33
48
11
Pneumonia arsenic,
sulfa alcohol
Liver biopsy
2
10
5
1
205
← Totals →
80

Fig 4 Diagram demonstrating the route of the thought processes in the differential diagnosis between medical and surgical jaundice, based primarily on laboratory examination (257) The numbers and the width of the individual lanes indicate the number of cases in each L, Tests for liver cell damage B, Tests for marked interference with bile flow.

surgical jaundice; (*c*) intrahepatic biliary "obstruction" in medical jaundice These rather common exceptions have clouded the interpretation of the hepatic tests

One attempt (257) to develop a system to overcome these difficulties (Fig 4) divides cases of jaundice, after eliminating the

hemolytic variety, into three groups: (1) those with evidence of liver cell damage and no interference with bile flow, (2) those without liver cell damage and with marked interference with bile flow, and (3) those with both To assume liver cell damage, at least two tests (not of the same type) should reveal abnormal results, in view of the relatively high incidence of "false positive" reactions One test with abnormal results suffices for presumption of marked interference with bile flow, provided obvious interfering factors, such as bone diseases (for serum alkaline phosphatase) or hypothyroidism (for total serum cholesterol), are ruled out. The cases of the first group are considered medical, except when an abdominal mass is present or when fluctuating urinary urobilinogen excretion on consecutive examinations suggests an incomplete extrahepatic obstruction Those of the second group are considered surgical, with the exception of the few cases in which a toxic factor is discovered or in which a preceding infectious hepatitis speaks for a cholangiolitic cirrhosis Cases of the third group can be subdivided into those with abnormal and those with normal flocculation tests (especially cephalin flocculation) The former group is considered medical, representing instances of primary hepatitis with some degree of intrahepatic "obstruction" The exceptions are either cases of purulent hepatitis in which chills, fever, or leukocytosis point to a septicemia, or cases with an abdominal mass The subgroup with normal flocculation tests is considered surgical (biliary hepatitis), with the exception of the few cases in which the history or liver biopsy findings suggest a toxic (medical) hepatitis The system just presented is subject to many improvements, but may represent a foundation upon which to build

(2) What tests aid in appraising the degree of liver cell damage or hepatic insufficiency in a jaundiced patient with an established diagnosis?

These tests are valuable in the observation of the course of hepatic disease, as a control of therapy, and for recognition of imminent hepatic failure. In general, the number of abnormal tests is important In principle, since residual function should be appraised, tests measuring reserve or capacity are preferable to those indicating activity, while those referring to dysfunction of other organs are obviously of little value The list of tests suggested for this

purpose by different authors varies; some (130) list bromsulfalein clearance, hippuric acid synthesis, prothrombin response to vitamin K, serum alkaline phosphatase, and galactose tolerance; others (245) recommend galactose tolerance and Millon's test, as well as determination of blood sugar and prothrombin time. We believe that the fluctuations in degree of hepatic insufficiency are best mirrored by cholesterol ester ratio, serum albumin or albumin–globulin ratio, bromsulfalein clearance, and hippuric acid synthesis or benzoyl glucuronate excretion, in the order listed, albumin being especially valuable in cirrhosis and the cholesterol ester ratio in obstructive jaundice Other tests, especially the flocculation tests, may serve similar purposes. In addition, the total serum protein level is a guide in the estimation of the dietary protein requrements

Tests indicating imminent liver failure (alarm signals) are: sudden rise of serum bilirubin, exceeding low hippuric acid synthesis (below 1 Gm in the oral test (129)), hypoglycemia, positive Millon reaction, and drop of a formerly elevated serum alkaline phosphatase; in addition, rise of the serum nonprotein nitrogen is especially valuable in obstructive jaundice and toxic hepatitis

(*3*) What tests are of value in the surgical management of hepatic disease?

A poor operative risk is indicated by reduced hippuric acid synthesis, marked bromsulfalein retention and hypoprothrombinemia resistant to vitamin K therapy When a malignant tumor is known to be present, hepatic metastases, which render the chances of surgical intervention poor, may be indicated by bromsulfalein retention (129) and other commonly used tests (1). Bromsulfalein retention occurs commonly following surgical operations (339).

Benign biliary obstruction is differentiated from the malignant type by a fluctuating, in contrast to a permanently suppressed, urinary urobilinogen excretion, by the presence of calcium bilirubinate and cholesterol crystals, and by the presence of many leukocytes or even purulent material in biliary drainage (130) The presence of blood suggests a malignant obstruction.

(*4*) What tests are of value in nonjaundiced or only slightly jaundiced patients?

For recognition of early (preicteric) or nonicteric viral hepatitis, early toxic hepatitis, and cirrhosis, the following findings are used

for screening purposes: bromsulfalein retention, bilirubinuria, cephalin flocculation, and increased thymol turbidity and flocculation, urinary urobilinogen excretion, and prompt-reacting serum bilirubin For the control of therapy with hepatotoxic drugs, the following tests are helpful: bromsulfalein clearance, hippuric acid synthesis, and determination of urinary urobilinogen, total and particularly prompt-reacting bilirubin.

(5) What tests are of value in animal experiments?

In larger animals, such as dogs, cats, and rabbits, in which blood and urine collection presents little difficulty, the same tests can be used as in man For the detection of hepatic damage produced by drugs, diets, or operative procedures on the biliary tract, the dye tests such as the bromsulfalein (68,69,130,221) and rose bengal clearance appear most reliable (338). Serum alkaline phosphatase (68,69,124) and prothrombin time (69) are slightly less sensitive (338). The intravenous galactose (69) and bilirubin tolerance tests (221,338) and determination of urinary urobilinogen reveal less consistent results (338). Urinary and blood bilirubin (18), icterus index, albumin-globulin ratio (338), total serum lipids (383), cholesterol ester ratio (342), and thymol turbidity (34) have also been used.

In rats and mice, satisfactory results have been obtained with an adaptation of the bromsulfalein clearance (43,300) Serum bilirubin determinations (43) seem helpful, whereas, unsatisfactory results are reported with thymol turbidity (43) Serum alkaline phosphatase has been determined in rats (244,374), as has total protein including albumin-globulin partition (21) Modifications of the hippuric acid test have been described for rats as well as larger animals However, variable results were obtained, apparently related to species differences (222). Marked cephalin flocculation is found in healthy animals of every species examined, rendering the test unusable in animal experiments

(6) What is the significance of biologically "false positive" and "false negative" reactions?

All hepatic tests occasionally reveal abnormal results in the absence of hepatic disease *In Tables I to VIII, the attempt was made* to list those without liver disease in three groups (*1*) normal subjects (usually students, nurses, and doctors), (*2*) control patients

(suffering from conditions without any known influences on the liver, as hernias, fractures, etc.), and (3) miscellaneous (chiefly patients with internal diseases in which some involvement of the liver cannot be excluded). It appears that in the normal subjects the results of the hepatic tests are abnormal in at least 2 5 per cent of the cases, with the exception of the colloidal gold test and cholesterol ester ratio which yield about 5 per cent abnormal results. In the 4 instances in which sufficient data are available, namely, the galactose tolerance, colloidal gold, cephalin flocculation, and thymol turbidity tests, about 10 per cent of the control patients reveal abnormal results. The miscellaneous group shows a high percentage of abnormal results in all tests; the cholesterol ester ratio reveals abnormal results in one-fifth of the cases; serum alkaline phosphatase and the thymol turbidity test in one-third; the cephalin flocculation and galactose tolerance tests in one-half; bromsulfalein, colloidal gold, and hippuric acid tests in two-thirds. These quantitative relations are incidental, since the material collected by the various investigators is not homogeneous in character. This miscellaneous group is not often a source of error, for the primary disease may be established by other clinical and laboratory procedures This group includes the following conditions which are known to influence liver function, infectious diseases of all types, especially pneumonia, malaria, septicemia, and lymphopathia venerea; rheumatoid arthritis and rheumatic fever; shock; hyperthyroidism; gastrointestinal disorders; renal diseases; tuberculosis, and even alcoholism and nonspecific fever reactions (104). Some of the abnormal results are explained by serum protein changes, especially in infections and renal diseases, and/or the presence of a clinically inconspicuous toxic hepatitis to which reference has previously been made. The large number of abnormal results in the miscellaneous group is therefore neither surprising nor does it detract from the value of the test, because it will seldom cause a differential diagnostic problem. If the number of positive results in the control group is added to the few in the miscellaneous group in which differential diagnosis has become a problem, abnormal results of the hepatic tests may be expected in at least 10 per cent of patients without liver disease This group represents a formidable diagnostic problem which can partially be overcome by performance of

several hepatic tests, preferably some of different physiologic bases

Similarly, a review of Tables I to VIII reveals that no test is abnormal in 100 per cent of the conditions in which liver damage is firmly established by clinical examination or other laboratory procedures. Roughly, in at least 10 per cent of instances with established liver cell damage, results of individual hepatic tests may be normal. The source of error due to these "false negative" reactions is again best excluded by performance of multiple tests

(7) What is the minimal number of tests recommended for the routine study of hepatic disease?

Obviously, every clinician and investigator may answer this question in a different fashion and there are many opinions as to a minimal but adequate program for diagnosis of liver disease (304). In summary of the opinions expressed in this review, the following are recommended to establish the presence of liver cell damage: the cephalin flocculation, thymol turbidity and flocculation and zinc sulfate turbidity, albumin–globulin ratio, urinary urobilinogen and bilirubin excretion, hippuric acid synthesis (or glucuronate excretion), and bromsulfalein clearance. In addition, for estimation of the degree of liver cell damage, primarily in obstructive jaundice, the cholesterol ester ratio or hippuric acid synthesis tests are suggested To determine the presence of marked interference with bile flow, the following are advocated· determination of urinary urobilinogen, serum alkaline phosphatase, and total cholesterol Total protein determinations are useful in the control of nitrogen nutrition, and those of total serum bilirubin for the general follow-up The hepatic tests just discussed, to which some clinicians may like to add aspiration biopsy of the liver and duodenal intubation, should not represent an excessive burden for a hospital laboratory

Summary

✓ None of the hepatic tests available measure a single basic function of the liver, and therefore no test or combination of tests will mirror its true functional status In addition, dissociation of the liver function explains why abnormal results in the different tests do not occur simultaneously. No test, even in severe liver damage, shows abnormal results in 100 per cent of the cases ("false negative" reactions). Furthermore, abnormal results in at least 10 per

cent of patients without apparent liver damage are encountered with almost every test ("false positive" reactions). Yet, marked progress has been made in the past 10 years in the practical use of the hepatic tests. This progress is due to a better understanding of the physiologic bases of the available tests, the development of more reliable and technically simpler proceedurees, the use of liver biopsy. and especially, to the tentative development of systems in the practical use of the tests. Such systems, founded on a comprehension of the physiologic bases of the tests, can be derived from correlations with morphologic and functional phenomena, independent of the disease, and where possible, with disease entities themselves This should eventually lead to a practical system in which each test is set in its proper place and in which the performance of multiple tests may permit the addition of probabilities derived from single tests By doing so, even nonspecific tests with frequent "false positive or negative" reactions may yield valuable information. Nevertheless, it should be stressed that the hepatic tests will not and should not replace clinical observation and judgment. Future progress lies both in the development of simpler, more sensitive and more specific tests, and, even more, in the better evaluation and systematization of the available tests. The latter point of view, which is the primary responsibility of the clinician, has been emphasized in this analysis

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cent of patients without apparent liver damage are encountered with almost every test ("false positive" reactions). Yet, marked progress has been made in the past 10 years in the practical use of the hepatic tests. This progress is due to a better understanding of the physiologic bases of the available tests, the development of more reliable and technically simpler procedurees, the use of liver biopsy, and especially, to the tentative development of systems in the practical use of the tests. Such systems, founded on a comprehension of the physiologic bases of the tests, can be derived from correlations with morphologic and functional phenomena, independent of the disease, and where possible, with disease entities themselves. This should eventually lead to a practical system in which each test is set in its proper place and in which the performance of multiple tests may permit the addition of probabilities derived from single tests By doing so, even nonspecific tests with frequent "false positive or negative" reactions may yield valuable information Nevertheless, it should be stressed that the hepatic tests will not and should not replace clinical observation and judgment. Future progress lies both in the development of simpler, more sensitive and more specific tests, and, even more, in the better evaluation and systematization of the available tests The latter point of view, which is the primary responsibility of the clinician, has been emphasized in this analysis

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The Vascular Physiology of Hypertension

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Eighteen years ago, to a youthful and self-confident eye, the problem of hypertension seemed a relatively simple one to be solved by the application of the experimental method and physiologic principles The following account will reveal that the writer still does not know the answer to most of the important questions regarding hypertension, in particular how it happens and why it happens in so many human beings. The reader hoping to have these questions answered or hoping to find a new hypothesis of hypertension will be disappointed. What the writer has tried to present is a critical appreciation of some of the facts that have been already established and their bearing on the chief problems at issue, in the hope that a reader may see a new ray of light. For the present impasse in hypertension is probably chiefly due to the fact that certain fundamentally important aspects of vascular behavior are appreciated by contemporary science either dimly or not at all.

In a problem as difficult as this it is particularly necessary to preserve certain rules of clear thinking. First, to relate the conclusion to the evidence on which it is based, to judge these conclusions as more or less securely established according to evidence, and to refuse to accept as fact anything which is little more than opinion, however distinguished its source Second, to avoid transferring conclusions, established under one condition or set of circumstances, to another that may be entirely different. Thus it is necessary to state again that hypertension is no more than a symptom, and that it may arise in more than one way It is quite possible, and indeed is known, that the operative factors elevating the arterial pressure in man are different in different diseases. And it is possible that essential hypertension, a group of cases largely isolated by exclusion, may comprise more than one entity. Again it is

necessary to draw attention to the danger of transferring to one species conclusions that have been established in another. So far as the human problem is concerned, a final answer can only be obtained in man.

It seems that, given the right experimental conditions, a lasting hypertension can be produced in most individuals of most species of mammalia. There is also no reason to suppose that more than a fraction of human subjects would be immune from hypertension, given the right conditions of disease. It is probable then that in many instances, if not in most, hypertension is due not to the operation of entirely new factors, but to factors that are present, though operating at a different level, in the normal animal. A brief review of the factors controlling normal arterial pressure may first be given.

Regulation of Arterial Pressure in Normal Animal

According to the principles of hydrodynamics, the pressure in the aorta and large arteries is determined by the cardiac output, the viscosity of the blood, and the frictional resistance offered by the systemic blood vessels. The cardiac output seems to be controlled very largely by vascular factors, for as Starling established in the heart-lung preparation, it is chiefly determined by the inflow from the great veins. For this reason, and because it is now known that in the commoner forms of human hypertension cardiac output is relatively normal, we need not dwell longer on it. Nor need we discuss blood viscosity, for its changes have little to do with hypertension. Our chief concern will be with the peripheral resistance and with the small arteries, arterioles, and capillaries that are the vessels chiefly contributing to it.

VASOMOTOR NERVES

The vasomotor nerves are believed to be of two kinds, adrenergic and cholinergic. Adrenergic nerves act by release at their endings of either epinephrine (adrenaline), or a substance like it such as noradrenaline (46). To most organs they are constrictor, but to the heart they are dilator. Anatomically they comprise the bulk of the postganglionic vasomotor fibers in the sympathetic nerves. Cholinergic nerves act by releasing acetylcholine or a close relative at their motor endings, and are largely vasodilator. Anatomically they comprise the preganglionic fibers of the sympathetic system, and the bulk of the parasympathetic vasomotor nerves.

The influence of the sympathetic nerves is very different, qualitatively and quantitatively, on the various tissues. Thus stimulation of the cervical sympathetic nerves produces intense vasoconstriction in the ear arteries and arterioles, but a very slight constriction in the arteries of the pia (47) (Fig. 1). Again, paralysis of the vaso-

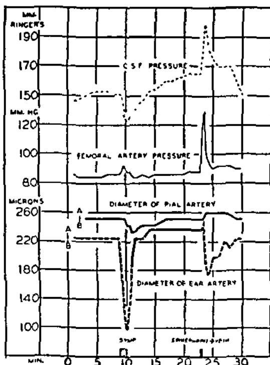


Fig 1. Effect of stimulating cervical sympathetic nerve and of epinephrine on diameter of an artery of the pia mater and an artery of the ear in the monkey under dial anesthesia (47) On stimulating the cephalic end of the cut cervical sympathetic nerve, the pial artery constricted 7 per cent and the ear artery 56 per cent Twelve minutes later, 1 cc of 1 100,000 solution of epinephrine was injected into the femoral vein, pial artery dilated 4 per cent, while the artery in the ear dilated 5 per cent and then promptly constricted 26 per cent

motor fibers will increase the rate of blood flow through a finger from 0 02 to 12 cc. per hundred cubic centimeters per minute (159), but raises blood flow through the forearm muscle only from 4 to

7 cc. per minute (11). Spinal anesthesia which paralyzes the vasomotor nerves has little influence on renal blood flow or tone of the glomerular vessels (145). Although the experiment has never been performed, it would seem from the facts just described that a generalized stimulation of the sympathetic nerves, such as has been conceived to be the mechanism of certain forms of hypertension, would have very unequal effects on the vascular supply of different tissues, and would lead to a great change in the distribution of blood in the body. We should expect the blood flow to be greatly diminished to skin and gut, and increased to brain and heart.

The experiments familiar to every student, of the hot flushed ear after section of the cervical sympathetic, and the steep rise in arterial pressure on splanchnic stimulation, perhaps exaggerate the importance of the sympathetic in the control of vascular tone; for, as we have seen, the effects in other territories are less marked. We are becoming more conscious of the possible importance of nonnervous factors, particularly as a result of experiments in which the sympathetic chains have been completely removed Thus it has been shown by Cannon (27) and by Bacq, Brouha, and Heymans (10) that in both cat and dog at rest, the arterial pressure returns to normal after complete removal of the sympathetic chains The sympathectomized cat is a poor creature, unable to take exercise, during which its arterial pressure falls, and unable to tolerate cold The sympathectomized dog, on the contrary, has no impairment of exercise tolerance and is able to run and fight as successfully as its normal fellow This behavior of the sympathectomized dog draws attention to the way in which both the general and local regulation of the peripheral resistance can proceed in the absence of the sympathetic, for it is inconceivable that a dog could behave as Heymans's dogs did unless arterial pressure and organ blood flow were reasonably well regulated during exercise The profound fall of blood pressure in the sympathectomized cat during exercise when contrasted with the rise of arterial pressure that occurs in the normal cat, is evidence of the importance of these nerves in that species at least

It is generally agreed that the cholinergic vasomotor nerves are of local importance only, and that they play little part in the regulation of arterial pressure So far as we know the chief importance

of the vasomotor nerves (dominantly sympathetic) is in the vascular reflexes, of which the chief are the carotid sinus and depressor reflexes to be discussed shortly, and the mechanism regulating body temperature which largely controls skin blood flow. The rises of arterial pressure in response to strong sensory stimuli, such as the rise on immersing the hand in cold water (cold pressor test) or on stimulating the central end of a sensory nerve, are chiefly of vasomotor origin, as are those in response to psychogenic stimuli

VASOCONSTRICTOR SUBSTANCES

Epinephrine. Rein (137) was the first to point out that epinephrine was not so much a pressor substance as a redistributer of blood. In man, in small quantities it has a profound constrictor effect on the vessels of the skin, which becomes pale and cold, on muscle vessels, however, its action is dilator (69, 71, 124) So that if forearm blood flow and hand blood flow are simultaneously measured, the one increases conspicuously while the other falls to very low values The systolic pressure rises while the diastolic remains unaltered or falls. The cardiac output rises, and the total peripheral resistance is decreased (113). Thus in small doses (single injections of 3–6 μg.) epinephrine does not increase the peripheral resistance Its action is rather to divert blood from skin to muscle and, in animal experiments, from gut to brain and heart Epinephrine is probably released in man during excitement, during the hypoglycemic attack, and during the performance of a mental problem (161). The nature of its action makes it highly improbable that it plays any part in the commoner forms of human hypertension In essential hypertension epinephrine can in fact be eliminated from the list of possible agents, for intravenous injection of a dose so small as to have no significant effect on arterial pressure will blanch the customarily red facial skin (129).

In medullary tumor of the adrenal gland (pheochromocytoma), paroxysms of hypertension with blanched cold skin occur, the arterial pressure between attacks being at first normal, later raised The attacks are cured by removing the tumor, which usually contains excessive amounts of what has been thought to be epinephrine Beer, King, and Prinzmetal (14) obtained from the blood of such a patient during an attack evidence of a substance which resembled

epinephrine in having a constrictor effect on the perfused rabbit's ear, abolished by ergotoxine.

The reader will note the discrepancy between the effect of epinephrine in small doses on the diastolic pressure and the diastolic pressures observed in adrenal tumor. It remains to be seen if this discrepancy is merely one of dosage or whether the substance in the tumor is not epinephrine. There is yet another discrepancy, namely, that the sympathetic apparently sends constrictor nerves to muscles, presumably adrenergic, yet epinephrine is dilator.

Posterior Pituitary Substance. This is a powerful antidiuretic substance. It has but a slight effect on arterial pressure when injected intravenously in man, but produces intense blanching of the mucous membrane and skin. Lewis (107), in man, and Heymans, Bouckaert, and Brouha (85), in the dog, have brought evidence to show that posterior pituitary substance plays very little part in the regulation of the normal circulation Its intense capillary action virtually rules it out as a major factor in the commoner forms of human hypertension.

Other Secretions of the Endocrine Glands. The most important of these appear to be the secretions of the adrenal cortex In Addison's disease the arterial pressure falls; adequate treatment with desoxycorticosterone acetate and salt restores it; overtreatment may produce hypertension (111, 150). The way in which desoxycorticosterone acetate and salt produce hypertension is not at all clear. The effect is not one that occurs within a few hours, as it is with most pressor substances; it is said not to be due to restoration of plasma volume and electrolyte levels (150) The effects of adrenalectomy in animals and of destruction of the adrenal by disease in man make it clear that the secretions of the gland play a part in the normal regulation of vascular tone. It is not beyond the bounds of possibility that they play a greater part in the mechanism of hypertension than we yet suspect

Renin. Renin has only been obtained from the cortex of the kidney But its precise origin there remains in doubt. Goormaghtigh (66) has suggested that the afibrillary cells of the juxtaglomerular apparatus secrete renin, and there is some agreement between the time relations of the hypertrophy and atrophy of these cells after renal artery constriction in the rabbit (66) and the renin content of

the kidney (134). Friedman and Kaplan (53) have concluded as the result of tartrate poisoning of the kidney that renin comes from the proximal convoluted tubules, but Govaerts, (67), in careful experiments, has been unable to confirm their findings. Earlier results on presence or absence of renin from kidneys of glomerular and aglomerular fish are not very convincing.

Renin is a protein and an enzyme. It acts on a constituent of the α-globulin fraction of plasma (hypertensinogen or renin substrate) to form a smaller molecule (hypertensin or angiotonin) Renin has not, as far as is known, any vascular action in the absence of its substrate It therefore seems that its pressor action is due entirely to hypertensin In speaking of the vascular effects of renin in this article, we speak in this sense.

The reaction of renin with its substrate hypertensinogen to produce hypertensin exhibits a certain degree of species specificity Thus, human renin will react with ox hypertensinogen, but ox renin will not react with human hypertensinogen. As is characteristic of enzyme actions, the amount of hypertensin formed in unlimited time depends on the amount of hypertensinogen in the original mixture and not on the amount of renin; the amount of renin determines the rate of the reaction; provided in both instances that hypertensinase, the enzyme destroying hypertensin, is absent from the mixture (26).

When renin is injected intravenously, the rise of arterial pressure begins about 30 seconds, and the peak is reached about 2 minutes after the injection; the response may last 30 minutes or longer (131) The response is believed to be due to the liberation of hypertensin and its subsequent destruction by hypertensinase Renin itself is fairly stable in blood at 37 C *in vitro* (92, 134); its gradual disappearance from the blood and the termination of its effect are presumably due to the action of the tissues, though which tissues acting in what way are questions as yet unanswered The response to an intravenous injection of hypertensin is much quicker and of shorter duration.

The rise of arterial pressure is chiefly or wholly due to vasoconstriction, the cardiac output being moderately raised in the animal (89) or remaining unchanged in man (22, 160). Tigerstedt and Bergman (151) showed that a rise still occurred in the pithed

animal, and vasoconstriction has been observed in isolated perfused preparations. Abell and Page (1) showed that in the rabbit's ear the chief vessels undergoing constriction were the arterioles, and we have observed the same In the rabbit the response differs from that to other pressor substances in that the skin temperature remains unchanged (102), and there is no paling of the ear or alteration of the central artery to macroscopic examination (131). Renal blood flow falls, but glomerular filtration rate rises, presumably an effect of efferent glomerular arteriolar constriction (34, 35). In man hypertensin produces a slight reduction in skin blood flow with no change in forearm blood flow (160). It seems, therefore, that renin produces hypertension by a rather uniformly distributed arteriolar constriction without greatly altering the distribution of blood, but the matter has as yet been insufficiently studied The point is important because, as we shall see, the distribution of vasoconstriction differs in the varying types of human hypertension, and the effect of renin seems, so far as present knowledge goes, to resemble the disturbance found in essential hypertension Renin does not raise the pulmonary artery pressure, and it is to be noted that this pressure is also not raised in experimental renal hypertension or apparently in human essential hypertension

We know that renin is released into the circulation when the renal artery is occluded, when the renal artery is clamped, and when the general arterial pressure is reduced by hemorrhage or histamine (80, 136, 140) It does not seem to be released by arterial anoxia (80). The most likely stimulus is thus an intrarenal pressure change, but it is not yet certain whether this is a fall in intravascular pressure (e g, in the glomerular tuft), or in pulse pressure as Page has suggested No evidence has yet been obtained that renin is secreted in response to nervous stimulation (26)

Because renin is a normal constituent of kidney and appears to be released by a change within the kidney, it would seem that its normal function in the animal economy is related to the working of the kidney Now its general effect and its renal effect have in common the raising of intravascular pressure within the glomerular tuft It has, therefore, been suggested (124) that the function of renin is to preserve intraglomerular vascular pressure on which glomerular filtration and the formation of urine depend It would

seem in fact that the renin mechanism is to be classed with the carotid sinus and aortic depressor reflexes as devices for the automatic control of arterial pressure. But while a fall of pressure in vessels supplying the brain or heart needs a very quick response, or else the animal will quickly lose consciousness or become weak, the consequences of a fall in pressure in the kidney are adequately met by a slower mechanism. The renin mechanism is a beautiful one for the purpose. For the substance, renin, released into the renal circulation is not itself active, and the actual vasoconstrictor substance, hypertensin, is only slowly produced in the general circulation. The renal vessels, therefore, get no more of the active agent than they would of one whose ultimate origin was from another organ. While this conception of the function of renin appears to fit most of the known facts, it ignores the extraordinary diuretic action of renin in the rabbit, where urine with a sodium and chloride content equalling those of plasma is secreted in large volumes (133). This effect, which is also obtained with hypertensin, is tubular in origin and is due to suppression of the mechanism responsible for the differential reabsorption of water and sodium and chloride (93a). The renin-hypertensin mechanism would thus seem to be a protection against a drop in arterial pressure, particularly in the kidney. Whether it plays any part in the maintenance of blood pressure in the normal resting animal is uncertain. It has not with certainty been detected in the renal vein in the normal animal. Removing both kidneys lowers the arterial pressure in the rabbit.

Biological Assay of Renin. Ten years have elapsed since renin was redescribed after its forty year sleep. During this period seven methods have been proposed for its biologic assay. These are summarized in Table I. The progress of science is very closely dependent on measurement, and it is quite clear from Table I that the measurement of renin can never be satisfactory until the definition of a unit is agreed to by all workers in the field and until the limits of error of the available methods have been defined. This is one of the more urgent problems in the field of hypertension, and attention is drawn to it here in the hope that some enlightened body may call a conference of workers in the field to reach agreement. Meanwhile, it is important to remark that a unit must, if possible, be defined in terms of weight of a particular substance, samples of which should be ob-

animal, and vasoconstriction has been observed in isolated perfused preparations. Abell and Page (1) showed that in the rabbit's ear the chief vessels undergoing constriction were the arterioles, and we have observed the same. In the rabbit the response differs from that to other pressor substances in that the skin temperature remains unchanged (102), and there is no paling of the ear or alteration of the central artery to macroscopic examination (131). Renal blood flow falls, but glomerular filtration rate rises, presumably an effect of efferent glomerular arteriolar constriction (34, 35) In man hypertensin produces a slight reduction in skin blood flow with no change in forearm blood flow (160). It seems, therefore, that renin produces hypertension by a rather uniformly distributed arteriolar constriction without greatly altering the distribution of blood, but the matter has as yet been insufficiently studied The point is important because, as we shall see, the distribution of vasoconstriction differs in the varying types of human hypertension, and the effect of renin seems, so far as present knowledge goes, to resemble the disturbance found in essential hypertension Renin does not raise the pulmonary artery pressure, and it is to be noted that this pressure is also not raised in experimental renal hypertension or apparently in human essential hypertension

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could be kept would clearly have advantages if the substance proved sufficiently stable. The definition of a unit in terms of its effect, adopted by a majority of workers in the field, is a very inferior method that has never been adopted for international use if a stable product is available. In the case of renin it may be quite fallacious, for in the unanesthetized rabbit the response varies from animal to animal and from day to day. In the anesthetized animal the response is also variable and complicated by tachyphylaxis, so that repeated injections are not always possible The direct and indirect methods of Leloir and his associates (26, 105) may prove to be the most satisfactory, complicated though they are, but here again an international standard is urgent, if work on the assay of renin is to be of permanent value, and if all workers are to speak the same language

Other Renal Vasoconstrictor Substances. Shipley, Helmer, and Kohlstaedt (144) have obtained in 2-day nephrectomized cats very prolonged pressor responses from the plasma of cats, in which the arterial pressure had been low for a long time due to infection, poisoning, or hemorrhage. The substance appears to be of renal origin, for it is not obtained from the plasma of nephrectomized animals similarly treated. Helmer and Shipley (81) have shown that the substance is more slowly destroyed by the nephrectomized animal than is renin.

Chambers and others (30) have shown that plasma from animals in the early stages of shock produced by bleeding or muscle trauma increases the frequency of spontaneous contraction and the response to epinephrine of the vessels of the mesoappendix. This vasomotor excitatory material appears to originate from kidney, but to have properties distinguishing it from renin and from hypertensin

VASODILATOR SUBSTANCES

Vasodilator substances are usually regarded as local rather than general regulators of vascular tone. Among them may be mentioned histamine, acetylcholine, adenosine and its relatives, nicotinic acid, kallikrein. The importance of such substances in the local regulation of vascular tone may be illustrated by reactive hyperemia and muscular contraction. As Lewis and Grant (109) showed, in amplification of the earlier observations of Bier and others, arrest of the circulation to a part results in the accumulation of vasodilator sub-

tainable from a central source. The alcohol-dried kidney powder first used as a standard had the advantage that it was easily prepared, and when kept dry and cold, was stable for at least two and

TABLE I

Methods for Biologic Assay of Renin

Author and Ref. no	Definition of 1 unit	Method of assay
Pickering, Prinzmetal (131)	Amount in 100 mg standard powder (alcohol-dried rabbit's kidney)	B P unanesthetized rabbit. Dose giving same response as standard Response not a linear function of dose, varies with different rabbits, not proportional to body weight
Hessel (83)	Amount raising B P. by 30 mm. Hg in 10 kg. dog under pernocton	B.P. dog under pernocton
Wakerlin, Chobot (154)	Amount raising B P. anesthetized dog by 40 mm. Hg	B P. response dog 3 hrs after nephrectomy
Swingle *et al.* (148)	Amount raising B P. anesthetized dog by 40 mm Hg	B P response anesthetized dog. Response a linear function of dose
Schale, Haynes (141)	Amount per kg. to give rise of 30 mm Hg in unanesthetized rabbit	B P response unanesthetized rabbit
Goldblatt *et al.* (62)	Amount raising B P. unanesthetized dog by 30–35 mm Hg in 3 min	B P. response of at least 3 unanesthetized dogs
Leloir *et al.* (105)	Amount producing 0 5 unit hypertensin when incubated at 37 C and pH 7 3, with excess hypertensinogen in absence of hypertensinase 1 unit hypertensin, amount raising B P. 10 kg chloralosed dog by 20–30 mm Hg. Standard kept	Production of hypertensin on incubation after destruction of hypertensinase Hypertensin assayed by finding dose of standard giving same response on B.P. of dog anesthetized with chloralose or nembutal

a half years This is a crude preparation, but a similar product was used as the international standard for posterior pituitary substance for many years A purer preparation of renin or hypertensin, prepared on a large enough scale so that an adequate number of samples

could be kept would clearly have advantages if the substance proved sufficiently stable. The definition of a unit in terms of its effect, adopted by a majority of workers in the field, is a very inferior method that has never been adopted for international use if a stable product is available. In the case of renin it may be quite fallacious, for in the unanesthetized rabbit the response varies from animal to animal and from day to day. In the anesthetized animal the response is also variable and complicated by tachyphylaxis, so that repeated injections are not always possible. The direct and indirect methods of Leloir and his associates (26, 105) may prove to be the most satisfactory, complicated though they are, but here again an international standard is urgent, if work on the assay of renin is to be of permanent value, and if all workers are to speak the same language

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stances, which are removed when the circulation is restored These substances appear to be constantly produced by most tissues (certainly muscle, skin, and brain), and in skin and muscle their rate of production is dependent on local temperature. This action is very powerful, for after a 5 minute circulatory arrest the forearm blood flow may rise from 3 to 40 cc. per hundred cubic centimeters per minute. The effect of these substances is to maintain organ blood flow, for reactive hyperemia accurately discharges blood flow debts accumulated during preceding circulatory arrest up to periods of 10 minutes. Blood histamine has been found to be increased in reactive hyperemia, but whether this is the active substance is still unknown.

Muscular exercise is accompanied by a considerable vasodilatation which in the forearm may raise muscle blood flow from 1 to 33 cc. per hundred cubic centimeters per minute, and which gradually declines over several minutes (69). This vasodilatation is undiminished in the sympathectomized limb and is probably of chemical origin. Anrep and others (6) have found a rise in the histamine content of the blood leaving the exercised muscles of dogs.

Several of these vasodilator substances exist normally in the blood, though probably chiefly in a bound or inactive form, and it is doubtful whether they are in general sufficiently stable or sufficiently active to affect vascular tone elsewhere.

Chambers and collaborators (30) have obtained evidence of the presence of a vasodepressor substance in the blood of animals in the later stages of shock

The action of these vasodilator substances, which are so important in regulating local vascular tone, may be largely responsible for the recovery of the arterial pressure and the remarkable range of activity of the sympathectomized dog In sum, they clearly play a very important part in the regulation of the peripheral resistance It would be by no means a farfetched hypothesis to suppose that a diminished production or accelerated removal of one or other of the more important of them (and I doubt if we are yet in a position to say which are the most important) might produce hypertension. Several attempts have been made to assay some of these substances in the blood and urine of patients with hypertension without very consistent or clear-cut results More directly relevant are experiments on reactive hyperemia The first experiments on forearm flow

showed essentially normal figures in essential hypertension for periods of arrest varying from one-half to ten minutes (122, 135a). Later experiments on muscular flow have shown rather greater flows in patients with hypertension (162), or no statistically significant difference (2).

MECHANISMS REGULATING ARTERIAL PRESSURE

That the arterial pressure is kept so constant in the normal animal suggests that there are mechanisms which detect changes in arterial pressure, and set afoot measures which counteract the change. The chief of these are the carotid sinus and aortic reflexes, our knowledge of which is chiefly due to the work of Hering (82) and Heymans, (86), whose books should be consulted for details. These reflexes start from stretch receptors in the adventitia of the aorta and carotid sinus, the nerve impulses ascending the depressor and carotid sinus nerves to the vasomotor center in the medulla. The impulses ascending the nerves are proportional to the stretch of the receptors and therefore to the intravascular pressure at these points. If arterial pressure rises, impulses are increased, the heart is slowed reflexly through increase of vagal and inhibition of sympathetic tone, and the blood pressure falls through inhibition of sympathetic vasomotor tone probably affecting all vascular territories, but only slightly brain and heart. If arterial pressure falls, impulses are diminished, the heart is quickened reflexly, and the blood pressure rises through increase in vasomotor tone and the release of epinephrine. In the dog, these reflexes depend very largely on the integrity of the sympathetic nerves, and no increase in arterial pressure follows section of all four buffer nerves after sympathectomy.

These reflexes are so important that they must be considered again, in relation to hypertension. It is possible that there are other but less important vasosensitive zones, as certain experiments by Heymans (84) suggest.

The other regulator of arterial pressure appears to be the renin–hypertensin system. But this seems to be less a regulator than a protector against a fall in arterial pressure. It is not, so far as we know, secreting during rest in the normal animal, and thus provides no protection against rise of pressure, as do the above mentioned reflexes.

Once again we may point out a lacuna in our knowledge. If these be the only, or even the chief, mechanisms regulating arterial pressure, how is it that the pressure returns to normal after complete sympathectomy in cat and dog?

Experimental Hypertension

Hypertension has been produced in animals by a variety of means, but here we shall deal only with two, that following section of the buffer nerves, and that following renal artery constriction. For a full review of earlier work, the reader is referred to Blalock's article (18).

HYPERTENSION FOLLOWING SECTION OF BUFFER NERVES

Hering and his pupils showed that section of the two aortic and two depressor nerves produced hypertension and tachycardia in rabbits and dogs. In the dog, hypertension is accompanied by an increased cardiac output and by increased blood flow through the limb (15). Nevertheless, peripheral vasoconstriction seems to be an important factor in the mechanism of the hypertension, for Heymans and Bouckaert (84a) have shown that hypertension persists after the tachycardia has been abolished by thoracic sympathectomy. The vasoconstriction is of sympathetic origin and is abolished by sympathectomy in the dog.

One of the striking features of this hypertension is its instability. The arterial pressure is much more susceptible than usual to splanchnic section, injection of vasoactive substances and hemorrhage (82, 86, 98). Strong sensory stimuli such as a prick, normally producing a rise of arterial pressure, are said to produce a fall in the rabbit with hypertension after section of the four nerves (98). Samaan (143) has made very important observations on the dog that deserve repetition and which indicate that the condition is not a continuous hypertension as we know it in the human subject. By making continuous records of pressure through an arterial canula, he found that the arterial pressure was normal when the animal was quiet or asleep, but quickly rose when the animal was approached, handled or otherwise excited. In fact, the feature of these animals is not so much persistent hypertension, as extreme lability of arterial pressure, resulting from the absence of the normal restraining reflexes. This would account for the absence of the arterial

lesions characteristic of malignant hypertension, despite the high levels of arterial pressure recorded

HYPERTENSION DUE TO RENAL ARTERY CONSTRICTION

By far the most important form of hypertension produced experimentally is that first described by Goldblatt and his colleagues (63) as occurring in the dog when the renal arteries were constricted by a silver clamp As Goldblatt (59) has pointed out, this form may follow a course resembling benign hypertension in man, with little or no impairment of renal excretory power for urea, with a stable hypertension, with cardiac hypertrophy, and with little or no vascular lesion except medial hypertrophy; or a course resembling malignant hypertension with papilledema and exudates in the fundus oculi, and widespread arteriolar necrosis It has been, and still is, expected by many workers that this form of hypertension will provide a clue as to the mechanism of hypertension in man But despite the great progress that has already been made, there is still a good deal of uncertainty as to the precise way in which this hypertension is maintained

Pattern of the Circulation Several observations have shown that, in the dog, cardiac output is within normal limits during this form of hypertension (91) Raised arterial pressure may therefore be attributed to increase in the peripheral resistance, and, since blood viscosity is unaltered, to vascular narrowing The pulmonary artery pressure is normal (94) In the early stages, and except in severe hypertension, when arteriolar necrosis develops, this vascular narrowing is functional, for histologic examination provides no evidence of organic vascular change other than hypertrophy of the arterial media This conclusion is supported by the reversibility of the hypertension When arteriolar necrosis develops, it presumably adds its effect to those of vasoconstriction, but a quantitative estimate of the contribution of each cannot be made

No observations are available to show precisely what order of vessels is involved in this vasoconstriction In the rabbit, the ear vessels, when dilated, either by sympathectomy or by warming the rabbit, appear normal, and the ear under these circumstances has the same order of skin temperature as it had before hypertension was induced (132). These observations suggest that the vessels nor-

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substance formed by normal kidney, because the slow disappearance of renin was not significantly accelerated in a 48 hour nephrectomized animal by replacement of 87.5 per cent of the blood volume by blood from a normal animal; and that the effect was due to uremia which altered the metabolic activity of the tissues destroying renin. Govaerts (68) has also shown that a nephrectomized dog develops over the course of 2 days a sensitivity to a pressor substance which emerges from a normal kidney artificially introduced into its circulation. Finally, total nephrectomy appears to sensitize the cat progressively to the slow-acting pressor substance of Shipley, *et al.* (144).

It therefore seems unjustifiable to conclude that the normal kidney eliminates, inactivates, or destroys the agent causing hypertension when the renal artery is constricted, though such an effect is not excluded by the facts available.

Nature of Disturbance in Kidney. Since the first paper of Goldblatt *et al.* (63), entitled "Production of Persistent Elevation of Systolic Blood Pressure by Means of Renal Ischaemia," it has been customary to refer to the affected kidney as the ischemic kidney, and to assume that the disturbance within the kidney which initiates the hypertension is a diminution in blood flow. That a conspicuous narrowing of the main arterial channel would reduce blood flow was a very natural assumption, and was supported by the measurements of Levy, Light, and Blalock (106), who found a 40 per cent decrease in flow in hypertensive animals 73 days after constricting the renal artery. There are, however, a number of experiments suggesting that the kidney has considerable powers of maintaining its rate of blood flow despite changes in the pressure of blood supplying it (48). Corcoran and Page (37) have published protocols of experiments in dogs; the clearances of phenol red and inulin were essentially unaltered in the animals in which hypertension developed after clamping of the renal artery. Alpert and Thomas (4) concluded from clearance observations that the renal blood flow may remain unaltered in hypertensive animals. Enger, Linder, and Sarre (45), Warthin and Thomas (155), and Schroeder and Steele (142) have also found that, after constricting the renal artery, the blood flow through the kidney may quickly return to its previous level unless the constriction is extreme.

Clamping the renal artery may also be expected to produce a fall

mally contributing most to the peripheral resistance, namely, the small arteries and arterioles, are chiefly involved in the vasoconstriction, and that the skin participates in this vasoconstriction neither more nor less than other territories.

Alleged Protective Action of Normal Kidney. Goldblatt (58) showed that in the dog constriction of one renal artery, the other kidney being intact, sometimes leads to hypertension, which is usually only moderate in degree and gradually subsides, but may be restored and intensified by excising the other normal kidney. It is very generally agreed that persistent hypertension is more certainly produced by excising one kidney and constricting the other renal artery or by constricting both renal arteries These observations have been very widely interpreted as indicating that the normal kidney has a protective action in that it neutralizes, destroys, or excretes the agent responsible for hypertension.

The observations, however, admit of other interpretations. Thus, in the rabbit, constriction of one renal artery, the other kidney being intact, may produce transient hypertension, but the affected kidney shrinks while the other hypertrophies It is evident that the normal kidney has taken over the work of the clamped kidney which has atrophied and that this process has been associated with the decline of hypertension It was therefore suggested (132) that the stimulus to hypertension might be related not only to the circulatory disturbance imposed by the clamp but also to the demands made on the kidney for its work In the dog, Verney and Vogt (152) have demonstrated the importance of the renal load by showing that hypertension produced by a clamp on the renal artery could be accentuated by administration of meat, sodium chloride, or urea

Rodbard and Katz (138) found, in dogs, that after excising the kidney when the renal artery had been constricted, the hypertension was abolished more quickly when the animal possessed a normal kidney than when it possessed none This experiment has been interpreted as proof of the hypothesis Rodbard and Katz's observations admit also of a different explanation Tigerstedt and Bergman (151), in their original paper on renin, showed its effect to be increased by nephrectomy Houssay and co-workers (92) observed that the rate of disappearance of injected renin was progessively slowed in the 48 hours elapsing after total nephrectomy They concluded that this effect was not due to the gradual elimination of a

conclusive Thus it has been shown that if the kidney is grafted into the neck (19) or groin (56), constricting its arterial supply produces hypertension which is abolished by removing the clamp on the kidney. Since there is no doubt at all that the connections of the kidney with the central nervous system were severed by these procedures, there can be little doubt that the way in which the kidney initiates hypertension in the dog is humoral; though it is to be noted that only short hypertensions have been proved in this way Further, it has been abundantly proved that hypertension can be produced and maintained in the totally sympathectomized dog (50, 87). It is certain, therefore, that constricting the renal artery in the dog can initiate and maintain hypertension without the aid of the chief vasomotor nerves

These experiments seem to prove conclusively that at least in the dog a humoral factor is responsible for initiating and maintaining hypertension. Nevertheless, there is some evidence which will be presented later to suggest that in chronic hypertension the nervous system may be concerned at least in part, though the writer considers this evidence to be inconclusive

Humoral Factors. The experiment of Blalock and Levy (19), showing that a rise of blood pressure follows constriction of the carotid artery supplying a kidney grafted into the neck, and that a fall to normal occurs following removal of the clamp, was the first quite conclusive evidence that under these circumstances a substance elevating arterial pressure is released from the kidney into the renal vein blood. The grafting experiments of Houssay and Fasciolo (93) had similar implications They showed that a kidney grafted into the neck of a dog whose kidneys had been removed 2 hours previously raised the recipient's pressure in 22 out of 24 instances when the kidney came from an animal with hypertension of 2 to 40 days' duration; but in only 4 of 25 instances when the source was a normal dog. The experiments of Grimson, Bouckaert, and Heymans (73) and the experiments of Govaerts (68) were less conclusive; and indeed O'Connor, Verney, and Vogt (118) show that a pressor substance is quickly released from a normal kidney when its circulation is even briefly interrupted in the operation of transferring it from one circuit to another Houssay and Taquini subsequently showed that blood, removed from the renal vein of hyper-

in arterial pressure distal to the clamp. Mason, Robinson, and Blalock (114) punctured the artery of the explanted right kidney distal to the clamp in unesthetized dogs They found that in 14 out of 15 hypertensive dogs the pressure in the artery distal to the clamp was less than before the constiction, and was always considerably less than femoral artery pressure, to which, in the hypertensive animal, it was not directly related

Finally, it has been suggested by Corcoran and Page (37) that diminution in pulse pressure is the effective stimulus, citing in support experiments on the perfused kidney in which reduction in pulse pressure leads to a development of a vasoconstrictor action by the effluent blood in the perfused rabbit's ear (99). Perfusion of the rabbit's ear as a method of detecting renin in the blood has, however, severely criticized by Landis *et al.* (103), who have pointed out the numerous uncertainties in the method.

It seems clear, therefore, that the facts do not yet permit of a final answer as to the nature of the stimulus to the production of hypertension imposed by renal artery constriction My own view, for what it is worth, is that the effective stimulus is a fall in intravascular pressure somewhere near the glomerular tuft It is fair to add that the subsequent rise in systemic arterial pressure may be expected in itself to diminish the effects of the clamp on the renal circulation, a consideration which may in part explain the difficulty in arriving at a conclusive answer

Neural Factors Denervating the kidneys has been found to prevent hypertension in the rabbit induced by oxalate (7), by nephritis from anti-rabbit kidney serum (8) and intrarenal injection of kieselguhr (112) In rabbits with hypertension of some weeks duration due to constriction of the renal artery of the only remaining kidney we failed to alter the arterial pressure by excising all visible nervous strands from the renal pedicle (128) This is the sole evidence, and it is not conclusive, excluding a nervous reflex in the rabbit In the rat there is no definite evidence on this point In the dog, denervation of the kidney has been said to prevent hyptertension due to constriction of the renal vein (24), and that due to intracisternal injection of kaolin (23) The evidence that a nervous reflex from the kidney is not involved in the hypertension due to renal artery constriction, or is at least of secondary importance, is

sion, arterial pressure slowly fell, reaching normal in about 4 hours Subsequently Blacket, Sellers and I (12c) were able to maintain hypertension with renin for 2 weeks with no sign of loss of response. There is no doubt that a prolonged hypertension can be produced by infusion of renin. We have had failure through using doses that were too large or too small, and with infected preparations of renin Provided these errors have been avoided, we have never failed to produce continued hypertension by prolonged infusion. The gross appearance of the animal and particularly the state of the vessels in the flushed ear in renin hypertension is indistinguishable macroscopically from the normal, as it is in hypertension from renal artery constriction.

The first publication throwing doubt on the renin hypothesis was that of Taggart and Drury (149), who showed that when enough renin was given to produce tachyphylactic extinction of the response to intravenous injection, the level of arterial pressure in a hypertensive animal was unchanged. They argued that since hypertension persisted while the vascular system had ceased to respond to renin, the hypertension could not be due to that substance Taggart and Drury did not mention in their paper how long their rabbits had had hypertension, but Dr Drury later informed me that it was of some months duration We had begun in 1936 the assay of renin in kidneys of rabbits with renal artery constriction and hypertension The assays were completed in 1939 and published in 1942 (131) In normal rabbits the renin content of the kidneys showed considerable variation, being greater in young rabbits than in old, but otherwise not related to factors we were able to control In a given rabbit, the two kidneys had equal renin contents within the limits of error of our method. Our largest series of results was obtained in animals from which one kidney had been removed; some of these animals were used as controls, others had hypertension from renal artery constriction (Fig 2). The following changes were found. (*1*) In animals in which constriction was so severe as to produce partial infarction of the kidney with hypertension lasting up to 48 hours then falling till the animal died, renin content of kidney was *very low* (*2*) In animals with hypertension of up to 8 days' duration, kidney not infarcted, the renin content of the kidney was increased (*3*) In animals with hypertension of over 2 months'

tensive dogs, when diluted with Ringer's solution had constrictor properties on the Lawen-Trendelenberg frog preparation, and that renal vein blood from normal dogs had not (26). Braun-Menendez and co-workers (26) showed that the pressor substance in renal vein blood was soluble in acetone and stable to heat. They called it hypertensin Now it had been expected that the active substance would be renin, for the existence of this, first demonstrated by Tigerstedt and Bergman, had in 1938 been independently confirmed by three groups of workers (83, 102, 131). But renin is insoluble in acetone and destroyed by heat. Braun-Menendez and his colleagues were thus led to incubate renin with serum and were able to isolate hypertensin from the mixture They were thus led to the conception, independently and simultaneously developed by Page and his colleagues, that renin was an enzyme. The same group (105) finally showed that if renal vein blood from a hypertensive animal was taken into an iced receiver, renin and not hypertensin was found in it, while no renin was detectable in normal blood Thus it is in fact renin that the clamped kidney secretes into the blood; but unless precautions are taken to prevent the reaction, this rapidly acts on hypertensinogen to produce hypertensin Thus it seemed quite clear that hypertension resulting from renal artery constriction was due simply to the release of renin by the kidney into the blood traversing it. While there seems to be no reason to doubt that this represents at least part of the truth, it is probably not the whole of it, as subsequent developments have shown We may now consider the evidence relating to the renin hypothesis in the various species in which the problem has been examined

We may begin with the rabbit It was in this species that Tigerstedt and Bergman (151) first demonstrated renin and observed that repeated injections led to reduction and finally extinction of the response Some, therefore, have doubted whether renin was capable of producing prolonged hypertension Prinzmetal and I (131), however, did observe that if sufficient time were allowed between injections for the arterial pressure to become normal tachyphylaxis did not develop Miss Hill and I (88) were able further to show that a continuous intravenous infusion of renin at constant rate would raise the arterial pressure by 30 to 40 mm Hg and maintain it there for the 4 hours the infusion was continued; on stopping the infu-

sion, arterial pressure slowly fell, reaching normal in about 4 hours. Subsequently Blacket, Sellers and I (12c) were able to maintain hypertension with renin for 2 weeks with no sign of loss of response There is no doubt that a prolonged hypertension can be produced by infusion of renin. We have had failure through using doses that were too large or too small, and with infected preparations of renin Provided these errors have been avoided, we have never failed to produce continued hypertension by prolonged infusion The gross appearance of the animal and particularly the state of the vessels in the flushed ear in renin hypertension is indistinguishable macroscopically from the normal, as it is in hypertension from renal artery constriction

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duration, the renin content of the kidney was normal. These results we were able to confirm by comparison with the clamped kidney and the previously excised normal kidney in the same animal. While the renin content of the kidney does not provide unequivocal information as to the rate of secretion of renin, these results suggested that the rate of formation of renin during the first week of hypertension might be increased, while after 2 months it might have returned to normal. Another chance observation led to

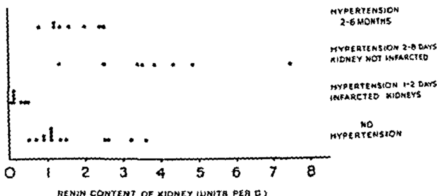


Fig 2 Results obtained from a series of rabbits in which one kidney had been removed, and the other kidney later assayed for its renin content (134) In those animals with no hypertension, the kidney was either intact or had had the renal artery exposed, but not clamped. In animals in which the renal artery was clamped and the hypertension lasted up to 2 days and then subsided, the kidney was found infarcted and contained little renin. In animals with maintained hypertension, the kidney showed an increased renin 2 to 8 days after clamping, and a normal renin content 2 to 6 months after clamping the renal artery

a similar conclusion For we found that in animals in which one kidney had been removed and hypertension produced by clamping the other renal artery (Figs 3–4), excising the ischemic kidney after 4 to 8 days restored the arterial pressure to normal in less than 24 hours in 7 out of 8 animals, while excising the ischemic kidney after 2 months left the hypertension unaltered during the 3 days the animals survived in all of 8 rabbits (126). The difference in behavior could not be attributed to alterations in a contralateral and previously normal kidney as it had been in the rat,

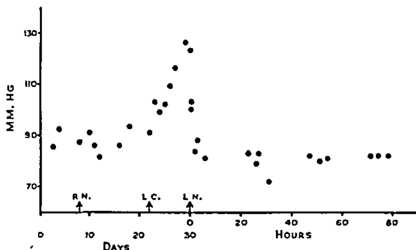


Fig 3 Rabbit 185 (126). Right kidney removed (R N) 10/17/38 Left renal artery clamped and kidney made subcutaneous (L C.) 10/31/38 Left kidney removed (L N) 11/8/38. Ordinate: arterial pressure Abscissa: time in days to left nephrectomy, then in hours Note that the arterial pressure returns to normal after the ischemic kidney is excised.

Fig 4 Rabbit 5B (126). Right nephrectomy (R N) 7/28/43 Left renal artery constricted (L C) 8/11/43 Left kidney removed (L N.) 10/7/43 Ordinate and abscissa as in Figure 3. Note failure of arterial pressure to return to normal after excising ischemic kidney.

for that kidney had been removed. Nor could it be attributed to arteriolar necrosis, for none was found; nor to progressive renal failure, for blood levels of urea before nephrectomy were normal The response to renin was found increased in rabbits with chronic hypertension, and to be further increased and prolonged by nephrectomy. Nevertheless, the persistence of hypertension in the absence of the kidney presented an inconsistency with the renin hypothesis, for (1) after intravenous injection of renin, the arterial pressure did return to its preinjection level in the chronically hypertensive nephrectomized rabbit; renin introduced into the blood stream was evidently eventually removed, yet hypertension persisted; (2) no source of renin other than the kidney could be found It seemed clear that the effects of removing the ischemic kidney during the first week were precisely in line with expectation if the hypertension were due to an increase in renin secretion by the kidney, as the assays had suggested. But the effects of removing the ischemic kidney at the end of 2 months showed quite definitely that a new factor contributing to the maintenance of hypertension had come into the picture, and that secretion of renin by the clamped kidney was not a sufficient explanation of the hypertension In fact, these experiments did more; they made it extremely doubtful whether increased secretion of renin by the clamped kidney plays more than an insignificant role in the maintenance of chronic hypertension; they are thus in keeping with the assays. Blackett and Sellers have since found that unclamping the renal artery after hypertension has lasted for 2 months will restore the arterial pressure to normal It is clear therefore that, whatever the new mechanism which comes to play such an important part in producing chronic hypertension in the rabbit, the mechanism is reversible and the primary cause lies in the clamped kidney, for clamping the renal artery produced it, and unclamping ended it In a previous paper, the new mechanism was referred to as a nonrenal factor. Looked at again 5 years later, to say that it is nonrenal would go beyond the evidence One possibility is of some substance retained in the body during the clamping of the kidney, and excreted or destroyed when the clamp is removed

To sum up, the evidence in the rabbit quite clearly suggests that during the first week after clamping the renal artery hypertension

is due simply to release of renin by the kidney; at the end of 2 months, the release of renin is small, and plays a less important part in the maintenance of hypertension than a new factor not yet identified

In the rat, Wilson and Byrom (164, 165) showed that a persistent and severe hypertension may be produced by constricting one renal artery. With this preparation they were able to show that acute necrotizing arteriolar lesions occurred in the unclamped kidney, but were absent from the clamped kidney, thus providing strong evidence for the suggestion made earlier by Wilson and Pickering (166), from observations on rabbits, that the chief factor in the pathogenesis of these lesions is the level of intra-arterial pressure attained These lesions in the unclamped kidney faithfully reproduced the renal lesions of malignant hypertension in man (164) Now Wilson and Byrom observed that if in such rats the clamped kidney were excised, the arterial pressure might fall to normal, or remain unchanged, or rise to higher levels From histologic examination of the unclamped kidney, they concluded that when there were few or no arteriolar lesions in it the arterial pressure fell to normal when the clamped kidney was removed; but when arteriolar lesions were pronounced in the unclamped kidney, hypertension persisted or was enhanced by removing the clamped kidney (165) Thus they were led to the concept, previously put forward by Volhard on clinical grounds, of a vicious circle in Bright's disease, of a hypertension which when severe enough led to renal ischemia, the renal changes leading to release of a pressor substance with further accentuation of the hypertension These observations were repeated by Friedman, Jarman, and Klemperer (52) who produced hypertension by wrapping one kidney in cellophane When this kidney was removed, the hypertension usually persisted though vascular lesions were not always found in the other kidney or elsewhere This hypertension they attributed to irreversible changes, perhaps related to the histologic lesions (hyalinization and necrosis) found in the vessels. Patton, Page and Ogden (119) also investigated the effects of excising the kidney whose artery had previously been constricted by silk In direct contrast to Wilson and Byrom, they found residual hypertension to be related to the duration of the preceding hypertension, but likewise attributed it

to arteriolar changes in the opposite kidney. Later Ogden and his colleagues (136) modified their views on the meaning of nephrectomy in the hypertensive rats, chiefly on the basis of the effects of nembutal and yohimbine. In rats made hypertensive by constricting one renal artery, the other kidney being intact, they found that hypertension of recent origin was unaffected by nembutal or yohimbine, but that long-standing hypertension was reduced or abolished by these drugs They therefore suggested that in the rat hypertension resulting from renal artery constriction is at first of humoral origin, but as time goes on a nervous mechanism becomes progressively and predominantly responsible for the maintenance of hypertension Although there is evidence, therefore, that with time there is a change in the mechanism of hypertension in the rat with renal artery constriction, the evidence as to its nature is not so clear cut as in the case of the rabbit, for the most direct experiment, namely, the effect of excising the ischemic kidney is open to two interpretations, and Wilson and Byrom's evidence is impressive The effects of nembutal and yohimbine are of interest, but can not be taken as proof of a nervous mechanism Nembutal has no constant effect on hypertension in the rabbit. That the central nervous system is concerned in maintaining chronic hypertension in rabbit and dog is also suggested by Dock and his colleagues (42), who found that destruction of the brain and spinal cord reduces the arterial pressure to the same low level in normal and hypertensive animals. Nevertheless, there is so much evidence of the initiation and maintenance of hypertension without the intervention of the nervous system that it is difficult to accept an idea based on such a severe procedure Recently, Braun-Menendez and his colleagues (25) have found no more renin in the renal vein blood of rats with chronic hypertension than in normal rats, and therefore suggest that release of renin is not the complete answer to chronic hypertension in this species

Hypertension has been more exhaustively studied in the dog than in any other species Goldblatt (58) showed very early that removing the ischemic kidney abolished hypertension even when it had been present for very many months, and this has been confirmed by many workers (19, 138, 152) While there has been no deliberate comparison between recent and prolonged hypertension, there is no

suggestion from the scattered results available that hypertension tends to persist after nephrectomy, even when the hypertension is of some months' duration. Nor are there available any assays of the renin contents of ischemic kidneys. The early results of Harrison and others (77) and of Prinzmetal and Friedman (135) were only roughly quantitative, and while suggesting an enhanced renin content were made chiefly on animals with recent hypertension The early observations, already referred to, of the Buenos Aires school on the presence of renin in the renal vein blood of animals with hypertension were made chiefly in animals whose renal arteries had recently been constricted. Thus Dell'Oro and Braun-Menendez (39) were unable to demonstrate renin in renal vein or femoral artery blood after excising one and explanting the other kidney. The renal artery to the explanted kidney was then clamped, 2 or 3 days later, after the arterial pressure had risen 20 to 40 mm renin was detected in both renal vein and femoral blood Very *similar amounts were found in the systemic blood of dogs in which* a similar rise of pressure had been produced by continuous infusion of renin. This experiment is the strongest piece of evidence that hypertension following renal artery constriction is due to the release of renin and to nothing else Assays have been conducted over a longer interval by Haynes and Dexter (79) They obtained rather irregular results which seemed to show that the amount of renin in the blood is maximal about a week after constricting the renal artery and then declines to a value that is not detectably abnormal Braun-Menendez *et al* (26) also agree that no excess of renin is demonstrable in the blood of dogs with considerable hypertension of 3 months' to 4 years' duration To sum up, it may be said that the evidence for the idea that the hypertension resulting from constricting the renal artery in the dog is due in the first instance to the release of renin from the kidney is extremely strong The evidence that hypertension of some years' duration is due to the release of renin is quite unconvincing To this extent the position in the dog resembles that in the rabbit, but there is not the same experimental evidence for a change of mechanism

Grollman and his colleagues (74, 75) take up quite a different position, holding that the chief factor in the production of hypertension is the partial or complete suppression of a substance formed

by normal kidney which is depressor in its action. They are prepared to admit that in its early stages hypertension is due to secretion of renin, but they believe that the other mechanism is particularly concerned with chronic hypertension. This view is based on the results which they have obtained from nephrectomy in rats, rabbits, and dogs When followed for a sufficiently long period, they claim that removal of a single kidney is as effective a stimulus to hypertension as is renal artery constriction or perinephritis. In chronic hypertension, removal of the clamped kidney in rabbit, rat, or dog does not abolish hypertension. It will be seen here that the issue is chiefly one of fact, for it has not been the experience of most workers that nephrectomy is an effective method of producing hypertension.

Lastly, mention should be made of antirenin. Wakerlin and collaborators (154) showed that when hog's kidney renin was injected into dogs, the recipient's serum developed the ability to neutralize the action of hog or dog renin. This is presumably an example of immunization to a foreign protein, the prosthetic group of renin acting as a haptene It is quite possible that with this new weapon, the renin hypothesis of experimental hypertension may be further probed. It has been shown that injection of large amounts of hog renin into dogs produces a high antirenin titer in the serum and may prevent hypertension from subsequent renal artery constriction, or reduce existing hypertension But opinion is still divided as to whether or not this is a specific antirenin effect (60).

Finally it is to be mentioned that Shorr *et al* (141a) claim that the phase of rising arterial pressure is accompanied by an increase in vasomotor excitatory material (V E M) in the renal vein blood and subsequently in systemic blood The phase of chronic hypertension reveals at first sight no rise in V E M , but further analysis shows an increase in both V E M and vasodepressor substance The evidence produced is one illustration that appears to be of a schematic kind Further and detailed evidence will be awaited with interest.

Influence of Endocrine Glands It has been shown in dog, rabbit, and rat that pregnancy does not accentuate, but, just before parturition, may reduce renal hypertension Removal of ovaries or testes does not affect hypertension

Removal of the whole pituitary, or of the complete anterior lobe, reduces the arterial pressure in normal animals, and has a similar effect in experimental hypertension. Anderson *et al.* (5) have reported that pituitary extracts containing the adrenocorticotropic hormone restore renal hypertension in rats to the level present before hypophysectomy, whereas extracts containing the lactogenic hormone fail to do so It is possible therefore that these effects of hypophysectomy are produced through the adrenal

Goldblatt (58) showed, in 1937, that in the dog adrenalectomy prevented hypertension from renal artery constriction, or abolished hypertension so induced previously. This observation has been abundantly confirmed, as has his earlier one that the medulla of the adrenal is not implicated The difficulty in interpreting these observations is that removal of the adrenals reduces the arterial pressure of normal animals and eventually leads to circulatory failure Is then the effect of adrenalectomy just the effect of removing the normal influence of the adrenal gland, or does it signify that the activity of the gland is altered in hypertension in such a way that it makes some active contribution to the hypertensive process? So far, no definite answer to this question can be given Goldblatt's earlier observations showed that adequate supporting therapy with sodium salts maintains life but not hypertension; the administration of cortin would restore some hypertension Collins and Wood (33), however, found that even in dogs receiving enough cortical hormone and salt to preserve hypertension the plasma sodium fell; they incline to the view that the gland does not behave abnormally in hypertension

Comment

It is now securely established that constriction of the renal artery, particularly in the absence of another normal kidney, will, if of the right degree, produce hypertension in a large variety of mammals, and that a similar effect has not as yet been obtained from any other organ. While it is not unlikely that there are minor differences, from one species to another, in the precise way in which the hypertension is produced and maintained, it is to be expected that in a broad general way the same kind of mechanism is stimulated in all animals There is now an impressive body of evidence that the mechanism is, at least in the early stages, humoral; that

by normal kidney which is depressor in its action. They are prepared to admit that in its early stages hypertension is due to secretion of renin, but they believe that the other mechanism is particularly concerned with chronic hypertension. This view is based on the results which they have obtained from nephrectomy in rats, rabbits, and dogs. When followed for a sufficiently long period, they claim that *removal of a single kidney is as effective a stimulus to hypertension* as is renal artery constriction or perinephritis In chronic hypertension, removal of the clamped kidney in rabbit, rat, or dog does not abolish hypertension It will be seen here that the issue is chiefly one of fact, for it has not been the experience of most workers that nephrectomy is an effective method of producing hypertension

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Influence of Endocrine Glands It has been shown in dog, rabbit, and rat that pregnancy does not accentuate, but, just before parturition, may reduce renal hypertension Removal of ovaries or testes does not affect hypertension

this condition is thus very imperfectly known and, in most instances, our acquaintance with it begins in middle life or later. From the time of Volhard, it has been recognized that the disease may follow two courses—benign and malignant. In the benign form, the patient is usually in the sixth decade of life when first seen and has a normal fundus oculi apart from retinal arteriosclerosis, or has what in England is called arteriosclerotic retinitis, in America, arteriosclerotic retinopathy; the renal function is normal or nearly so, the diastolic pressure is usually less than 130 mm Hg Such a patient's condition may remain unchanged for many years, and death when it occurs is due to apoplexy, cardiac failure, or intercurrent disease. In the malignant form the patient is usually younger and the diastolic pressure higher The first sign of an unfavorable course is found in the retina in the form of bilateral papilledema and ill-defined exudates, the picture of albuminuric retinitis, or hypertensive neuroretinopathy. The course is rapidly downhill; albumen and red cells appear in the urine, the kidney fails and death occurs usually within the year of uremia.

The relationship between benign and malignant hypertension has been discussed elsewhere (125), it is enough to say that hypertension in the two forms differs in degree but not in kind The evidence that arteriolar necrosis is due to high intravascular pressure is supported by experience in man, for the lesions are characteristically found in those with the grossest hypertension, and they may occur when there is no renal lesion Some authors, from their own experience with animal experiments, consider that renal insufficiency is a necessary factor in producing arteriolar necrosis Human experience agrees with other animal experiments in showing that arteriolar necrosis may occur when the renal lesion is minimal and there is no nitrogen retention in the blood.

Pathogenesis of Essential Hypertension It has long been suspected that heredity was an important factor in the pathogenesis of essential hypertension The first exhaustive enquiry into this point was that of Weitz (157), who found that of 82 outpatients attending for hypertension, 76 8 per cent had a family history of death from apoplexy or heart failure in one or both parents, while in 267 controls over 44 years old, attending for other disease (but of whom some had hypertension) 30 3 per cent had a similar family history

is to say, as a consequence of renal artery constriction, a substance producing generalized vasoconstriction is added to the blood as it traverses the kidney; and there is evidence that this substance is renin. It seems that as time goes on other changes occur in the animal, such that although the hypertension is maintained, the amount of renin actually being secreted by the kidney is less than it was earlier, and in fact may become so small that present methods are unable to detect it with certainty, and perhaps so small that it has no definite effect on arterial pressure. In this chronic stage, the release of renin into the circulation is thus probably not the only factor in maintaining hypertension It is still uncertain what the new factor or factors are that contribute to the raised arterial pressure in chronic hypertension Their identification is probably one of the most urgent problems in the whole field of hypertension today, as we shall see when we have discussed the problem of hypertension in man

Blood Vessels in Human Hypertension

Hypertension is a symptom found in a number of maladies that are clinically distinct It is possible that the vascular disturbance is different in these several kinds, and each must be considered separately Too little is known of the mechanism in many of these instances to justify discussion in this article

ESSENTIAL HYPERTENSION

Definition Essential hypertension is diagnosed by exclusion, by the absence of features characteristic of other conditions It may, therefore, comprise more than one entity During the last two decades two conditions have been recognized that would earlier have been classified under essential hypertension, namely Cushing's syndrome, and a group of cases presenting the histologic changes of pyelonephritis There may yet be others, and it may be that some of the discrepancies between the results of various authors are due to differences of material

Course The beginning of essential hypertension is shrouded in mystery in most instances, except for the occasional unfortunate college entrant in whom raised arterial pressure is discovered For in this age of propaganda in the name of truth, he may become a hopeless neurotic or be subjected to major surgery The early history of

this condition is thus very imperfectly known and, in most instances, our acquaintance with it begins in middle life or later. From the time of Volhard, it has been recognized that the disease may follow two courses—benign and malignant. In the benign form, the patient is usually in the sixth decade of life when first seen and has a normal fundus oculi apart from retinal arteriosclerosis, or has what in England is called arteriosclerotic retinitis, in America, arteriosclerotic retinopathy; the renal function is normal or nearly so; the diastolic pressure is usually less than 130 mm Hg. Such a patient's condition may remain unchanged for many years, and death when it occurs is due to apoplexy, cardiac failure, or intercurrent disease. In the malignant form the patient is usually younger and the diastolic pressure higher. The first sign of an unfavorable course is found in the retina in the form of bilateral *papilledema* and ill-defined exudates, the picture of albuminuric retinitis, or hypertensive neuroretinopathy. The course is rapidly *downhill; albumen and red cells appear in the urine, the kidney* fails and death occurs usually within the year of uremia.

The relationship between benign and malignant hypertension has been discussed elsewhere (125); it is enough to say that hypertension in the two forms differs in degree but not in kind. The evidence that arteriolar necrosis is due to high intravascular pressure is supported by experience in man, for the lesions are characteristically found in those with the grossest hypertension, and they may occur when there is no renal lesion. Some authors, from their own experience with animal experiments, consider that renal insufficiency is a necessary factor in producing arteriolar necrosis. Human experience agrees with other animal experiments in showing that arteriolar necrosis may occur when the renal lesion is minimal and there is no nitrogen retention in the blood.

Pathogenesis of Essential Hypertension. It has long been suspected that heredity was an important factor in the pathogenesis of essential hypertension. The first exhaustive enquiry into this point was that of Weitz (157), who found that of 82 outpatients attending for hypertension, 76.8 per cent had a family history of death from apoplexy or heart failure in one or both parents, while in 267 controls over 44 years old, attending for other disease (but of whom some had hypertension) 30.3 per cent had a similar family history.

He also measured the blood pressure of 93 brothers and sisters of 42 hypertensive patients. He argued that if this were a hereditary disease slowly unfolding with age, all brothers and sisters older than the patient should have developed hypertension or died of it if they carried the gene Restricting the enquiry then to this category, he found 11 older siblings dead, 20 with pressures above 150 mm. Hg and 27 with pressures below this figure. Now this is nearly a 1:1 ratio, and Weitz therefore suggested that essential hypertension is due to the possession of a gene that is transmitted after the manner of a Mendelian dominant. Weitz's conclusions have been largely confirmed by Hines (90) and Platt (120).

Circulation in Essential Hypertension

It can now be accepted that hypertension is due to vascular narrowing, for measurements of cardiac output (64) and blood viscosity (122) have yielded normal figures We have to decide what order of vessels is involved, how the narrowing is distributed over the tissues of the body, and its nature

There are too few direct measurements of intravascular pressure at various parts of the circuit to enable us to say with certainty which size of vessel is abnormally narrowed in essential hypertension But on general grounds it is suspected to be the small arteries and arterioles, and indirect measurements agree with this view.

It is also very generally agreed that organic vascular changes play a secondary and inconstant role in this vascular narrowing The various kinds of disease affecting the large arteries, such as atheroma, occur not only in patients with hypertension but also in those with normal blood pressure Atheroma is extremely important as being the chief cause of local ischemia, for example of heart and brain, and therefore one of the chief causes of disability and death in essential hypertension It occurs, however, though probably with lower incidence, in subjects of the same age and with normal arterial pressures Degenerative changes in the large arteries, in which muscle and elastic tissue are replaced with more rigid material, lead to increase in pulse pressure and thus in pure form to systolic hypertension Systolic hypertension should not be confused with essential hypertension, in which the diastolic pressure is raised also It is thus clear that these changes in the large arteries do not of themselves contribute to the increased peripheral resistance.

The part played by organic change in the smaller vessels is much more difficult to judge. From a study of the circulation as a whole, two reasons have been given for supposing that the abnormal constriction is not organic in nature, but neither of these reasons is unequivocal. The first is that, as Volhard (153) pointed out, and as has subsequently been confirmed, fever may reduce the arterial pressure in essential hypertension to normal levels, and this reduction is achieved not by fall of cardiac output, which may in fact rise, but by vasodilatation, the total peripheral resistance becoming normal (21). The fall in arterial pressure and of total peripheral resistance in response to fever is much greater in essential hypertension than in normal subjects; one may argue therefore that it is particularly the abnormal narrowing that is affected in the febrile reaction, and that this is consequently reversible and not organic The second reason is that vasodilator agents, such as histamine, give a larger fall of arterial pressure in hypertensive than in normal subjects, a fall approximately proportional to the initial arterial pressure (130) This again is more easily explained if the abnormal resistance is functional and not organic. Perhaps the most important evidence is histologic, for this has revealed that in early cases of essential hypertension arteriolar lesions may be slight or absent, even in their place of predilection, the kidney (28) In more advanced cases, and particularly in malignant hypertension, the vascular lesions in the small arteries and arterioles may be so numerous that they may play a part in the peripheral resistance; but even in such cases fever may reduce the arterial pressure to the normal range

Thus it would seem that, while structural changes in the small arteries and arterioles may contribute, they are not the chief factors in the increased prepiheral resistance which is the cause of the raised arterial pressure. The chief factor is evidently vasoconstriction which may be of nervous or humoral origin Its nature will become clearer when we consider the distribution of the vascular narrowing

Experimental physiology has taught us that the vessels of the body behave as a series of units in their response to certain reflexes affecting the circulation and in response to vasoactive substances Thus the skin vessels tend to behave similarly all over the body and in a way that is different from the vessels to muscle or brain when, for example, the body temperature is raised or epinephrine is in-

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While the skin circulation is thus not suited to demonstrating small variations in blood flow under resting conditions, it is, in the hand, admirably designed for investigating the nature of the agent narrowing the vessels, for sympathetic vasoconstrictor tone is easily removed by warming the body (110), and the main nerves to the hand are accessible to anesthetization.

Now there are three chief methods available for assessing the rate of blood flow through the skin Skin temperature can be at once dismissed as being incapable of detecting any significant change and as being too much influenced by other factors such as air movement and the rate of evaporation from the skin. The plethysmographic method of Hewlett and van Zwalenburg, in which the rate of expansion of the tissue enclosed in the plethysmograph is measured, gives flow in cubic centimeters per 100 cc. per minute It is capable of giving accurate results It is also capable of giving most inaccurate results and it is not always easy to distinguish one from the other. The chief sources of error are:

(1) When inflows are fast, the veins of the part quickly become distended and the slope of the curve gradually flattens The inflow in such cases is assessed by drawing a tangent to the curve in its earliest and steepest part. It is of course no more than assumption that the fastest rate of volume increase represents actual blood flow, and it is an assumption that can not be checked This objection may be minimized by keeping the part above the sternal angle and thus as empty of blood as possible.

(2) The blood may collect first in the tissue between the collecting cuff and the plethysmograph Thus, in estimating blood flow through a digit, occluding a cuff at the base of a digit may give a flow three times that obtained by occluding a cuff on the wrist (57) This error may be minimized by placing the cuff as near to the plethysmograph as possible

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jected intravenously. If the vascular narrowing in hypertension affected all vessels to a similar degree, then in the first place we should expect each organ to receive its normal quantity of blood, and in the second we should have to search for some agent which had a uniform action on different vessels as the cause of essential hypertension Alternatively, if the vascular narrowing were unequal, then blood would be diverted from organs in which the vessels were most narrow to those organs in which the vessels were wider; we should thus find some organs receiving less and some more blood than normal, and our agent should have a similar differential action on the vessels One further and important point can be learned from a study of tissue blood flow. If the vasomotor nerves are responsible for the abnormal vasoconstriction in hypertension; then if it were possible temporarily to eliminate their influence over one vascular territory while leaving the arterial pressure unchanged, the blood flow through that territory should rise to much higher levels than in normal people subjected to a similar procedure; for, the effective agent being removed, the vessels to the part would be reduced to the same state in normal and hypertensive subjects, and blood flow would be directly dependent on arterial pressure

Forearm. The first part to be investigated was the forearm Early in 1936, Prinzmetal and Wilson (135a), and I (122), independently, published measurements of the forearm blood flow by the plethysmographic method of Hewlett and van Zwaluwenburg, using Lewis and Grant's plethysmograph. We each found similar rates of blood flow in normal subjects and subjects with persistent hypertension, both at rest and in response to the vasodilator stimulus of circulatory arrest for 10 minutes In addition, Prinzmetal and Wilson found similar rates of flow in response to local heat and to warming the body or anesthetizing the cervicodorsal sympathetic ganglia with novocain For our present purposes these observations are largely of historical interest, and for two reasons. we did not know that the blood flows also represent in part blood flow to the hand, as Grant and Pearson (71) later showed, and the forearm consists of two tissues—muscle and skin—in addition to bone

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In Stewart's calorimetric method, the hand is immersed to a known point in stirred water in a calorimeter, the temperature changes of which are read. The method has recently been improved by Greenfield *et al.* (72). Now, the readings can not be used to calculate blood flow in cubic centimeters per 100 cc. per minute because the blood flowing through the hand is not reduced to calorimeter temperature but to some point above By introducing a needle thermocouple into the lumen of a vein draining the hand, however, it is possible to estimate this temperature and to find out whether it has differed in the two series of observations to be compared. For the temperature of the venous blood leaving the part and the temperature of the arterial blood entering the part which is in turn affected by venous blood temperature, are the only important sources of the error in the calorimetric method.

In the observations on the blood flow through the hand, the calorimetric method was chosen in preference to the plethysmographic one, because although the latter does admit calculations of blood flow its sources of error are much more difficult to control And since a comparison between blood flow in normal and abnormal subjects was required, it was more important that the results should be reliable than that they should be expressed in cubic centimeters per 100 cc Recent observations of Greenfield and Scarborough (72) have shown that if care is taken the results with the two methods agree well Disagreements of fact found by workers using the plethysmographic method may well be due to the uncontrolled technical error.

To remove vasoconstrictor nervous tone from the hand, body temperature was raised by immersing the opposite forearm in water at at 44 C Evidence will be found in the original paper to show that heat elimination provided a true reflection of skin blood flow in subjects with normal and raised pressures, and that the degree of body warming employed did in fact completely remove vasoconstrictor nervous tone from the hand vessels without stimulating vasodilator nerves and without significantly altering arterial pressure In this way it was shown that the blood flow through the skin of the hand from whose vessels vasomotor nervous tone had been removed was no greater, but sometimes a little less, in 15 subjects with benign and malignant hypertension than in 21 subjects of comparable age

with normal pressures Evidently, then, in essential hypertension the hand vessels are narrowed by a non-nervous agent, and this narrowing is of an order such that if generally distributed through the body it would account for the hypertension Since the skin vessels are rarely and inconsiderably involved in the arteriolosclerosis of hypertension, the narrowing is presumably not of structural but of humoral origin. Stead and Kunkel (146) obtained the same order of blood flow through the hand maintained in a plethysmograph at 43 C. as they did in normal subjects

Muscles Measurements of forearm blood flow, after excluding the hand, have been made plethysmographically by Stead and Kunkel (146), Wilkins and Eichna (162), and Abramson and Fierst (2) If we assume that the plethysmograph was placed as high on the forearm as possible, then Grant and Pearson's (71) investigations would indicate that 85 per cent of the tissue whose blood flow they measured was muscle. Abramson and Fierst found that with the plethysmograph maintained at 32 C the blood flow averaged 2 86 cc per 100 cc per minute in hypertensive subjects and 1 77 cc in normal ones Moreover, if the subjects were grouped according to their systolic pressures, it was found that the higher the arterial pressure the greater was the forearm blood flow Similar results were obtained in the calf. Abramson and Fierst unfortunately did not attempt to classify their subjects with hypertension, and we have to assume that their observations refer to essential hypertension, since this is by far the commonest condition From their observations it seems, therefore, that the muscle vessels are either unconstricted or less constricted relative to the other vessels in the body, so that at rest the muscles get more than their normal share of blood Observations on the effects of exercise on muscular blood flow are difficult to make truly quantitative, but, so far as they go, both Stead and Kunkel and Abramson and Fierst have found normal flows in hypertension Wilkins and Eichna observed flows after 5 minutes of circulatory arrest that were larger in subjects with hypertension than in those with normal arterial pressure, this was not found by Abramson and Fierst

Brain If it is asumed that the metabolism of the brain is the same under different pathogenic conditions, any changes in cerebral blood flow should be reflected by changes in the carotid-jugular O_2

and CO_2 differences. Williams and Lennox (163) have found the values in 21 patients with hypertension to be within the normal range. They also found normal values in cerebral arteriosclerosis and high intracranial pressure, and stress the importance of the local regulating effect of CO_2 on the blood flow through the brain Kety and collaborators (96), using their nitrous oxide method, have recently found identical rates of cerebral blood flow (54 cc/100 gm./min.) in normal subjects and patients with essential hypertension.

While it is therefore probable that the blood flow through the brain as a whole is normal in essential hypertension, it is widely maintained that from time to time the cerebral arteries show intense contractions leading to transient ischemia of the area of brain supplied. A careful consideration of the evidence and of my own experience in this and other conditions has convinced me that this hypothesis is unnecessary to explain these phenomena of hypertensive encephalopathy; the acute form, with convulsions and coma, is due to acute cerebral edema; the chronic form, with transient palsies, to cerebral thrombosis. My evidence against the hypothesis of vascular spasm chiefly depends on a series of cases of transient cerebral disturbances occurring in mitral stenosis and auricular fibrillation and undoubtedly due to cerebral embolism (127).

Retina. The retina has peculiar interest, for here, uniquely, vessels measuring from 20 μ diameter upward may be actually seen and their changes observed Before the invention of the sphygmomanometer, Gowers (68a) stated that the degree of constriction of the retinal arteries was proportional to the height of the blood pressure. Measurement has shown that the retinal artery diameter is reduced in hypertension, but has not revealed quite the precise relationship Gowers indicated (16) Friedenwald (51) has drawn attention to the frequency with which retinal vascular narrowing in old people is associated with atheromatous plaques on the central artery of the retina In a case of malignant hypertension, with as narrow retinal arteries as I have ever seen, the central artery was almost obliterated by arteriolar necrosis It is probable, therefore, that functional vasoconstriction is not the only cause of retinal arterial narrowing in hypertension With ever-increasing frequency internists and ophthalmologists speak of retinal arterial spasm,

often with respect to the localized constrictions that occur along the course of the arteries There are very few subjects in medicine which have invited quite so much loose thinking and loose talk as spasm. Those who have observed these localized constrictions carefully, speak of them as fixed, that is to say, that they remain unchanged in position and size for weeks or years, and such has been my own experience. They have in fact the characteristics of organic arterial changes, and many histologic examinations have supported this view. There are cases in which whole stretches of artery suddenly disappear from view to reappear later. There is no evidence that these cases are more frequent in hypertension, and their causation is very imperfectly understood My own view is that the occurrence of localized spasm of cerebral or retinal arteries must be very rare in essential hypertension, as I have never seen such a case in which the evidence would stand critical examination The question as to whether localized arterial contraction occurs in brain and retina is important because of the implication that there is a local cause of vasoconstriction On the principle of the paucity of hypotheses, this locally acting agent should be the agent responsible for hypertension

Kidney Our knowledge of the circulation through the kidney is largely dependent on the clearance methods, perfected and tested by Homer Smith and his school Although the methods for determining the glomerular filtration rate (inulin, mannitol, etc , clearances) and renal blood flow (diodrast, *p*-aminohippurate, etc , clearances) are indirect, there is now an impressive body of evidence as to their reliability except when there are profound disturbances in the animal In particular renal vein catheterization has enabled the extraction ratio of diodrast and *p* aminohippurate by the kidney to be determined and the one major assumption checked

By such methods, Goldring *et al* (65) have investigated the renal circulation in 60 cases of essential hypertension up to the terminal *malignant phase. A major difficulty in comparing normal* and hypertensive kidney is the frequency in the latter of organic vascular changes and their consequences To surmount this difficulty, inulin and diodrast clearances were related to the value for the total amount of functioning tubular tissue, as given by the maximal capacity of the tubules to excrete diodrast (Tm_D) They

found that the tubular excretory mass was reduced in all but 3 cases, and in the 8 patients in the malignant phase was between 6 and 38 per cent of the normal value. The effective renal blood flow inferred from the diodrast clearance was also reduced, being between 41.7 and 1176 cc. per minute; in 45 of 60 cases it was more greatly reduced than the tubular mass, suggesting that some factor is operating in hypertensive subjects to produce a relative ischemia in the residual functional tubular tissue. The rate of glomerular filtration is much less reduced than the tubular functions, so that the fraction of the renal blood flow filtered off in the glomeruli is increased: "Since the relative ischaemia in these subjects is correlated with a rise in filtration fraction we conclude that the ischaemia is the result of increased resistance beyond the glomeruli—i e, probably in the efferent glomerular artery."

Goldring, Chasis, Ranges, and Smith believe the constriction of the efferent glomerular arterioles to be of humoral origin, because it is not nervous and it can be relaxed. The vasoconstriction is not neural in origin, because in essential hypertension the renal blood flow is not increased, nor is the filtration fraction lowered, by operations in which the sympathetic nerves supplying the kidneys have been divided The vasoconstriction can be relaxed through the induction of fever by intravenous injections of pyrogens which, in essential hypertension as in normal subjects, increase the effective renal blood flow while leaving the filtration rate unaltered The tubular excretory mass is usually not increased by the pyrogenic reaction, but in some subjects with essential hypertension it rises significantly, suggesting that substantial amounts of tubular tissue may be ischemic under basal conditions This argument rests chiefly on the decreased ability of the kidney in essential hypertension to excrete diodrast I am, however, unable, after careful consideration, to see any satisfactory alternative to the explanation of their results adopted by these workers

It seems, then, that there are intrinsically two changes in the kidney in essential hypertension: first, a reduction in the amount of functional renal tissue, inferred to be progressive and accompanied by a corresponding reduction of renal blood flow, and secondly a relative ischemia of the remaining functional tubular tissue due to narrowing of the efferent glomerular arteries The first of

these changes has long been suspected by those working on post-mortem material and attributed to structural vascular lesions, especially of the afferent glomerular arterioles Smith and his colleagues also consider that the efferent glomerular vasoconstriction is an effect of the process responsible for hypertension rather than in any sense its cause—a conclusion supported by Chasis and Redish's (31) observations that vasoconstriction usually affects equally the two kidneys in essential hypertension.

Circulation as a Whole. In 1943 figures were collected to show how the cardiac output was distributed in a patient with essential hypertension, as compared with a normal subject of similar size and under similar conditions of rest. Table II shows such a comparison, in which the figures have been revised in the light of newer and more accurate methods of measurement. Too much attention should not be paid to detail in what is a compilation of figures obtained from different material. But the figures suggest strongly that despite the considerable rise in arterial pressure in essential hypertension, and the corresponding vasoconstriction, the various organs of the body receive amounts of blood that are very similar to those in subjects with normal pressure The abnormal vasoconstriction is thus distributed with remarkable uniformity over the tissues of the body, sparing the muscles a little and being unusually intense in the kidney. In seeking the cause of this vasoconstriction, it would seem wise to look with particular care for an agent that can cause a vasoconstriction of this distribution From what has been said earlier, it will be clear that the pattern of vasoconstriction is quite unlike that imposed by epinephrine, and rather unlike that which overaction of the sympathetic nerves would cause; it is quite like that resulting from the action of renin and hypertensin But there probably are other ways, at present imperfectly appreciated, in which this can happen

Cause of Vasoconstriction in Essential Hypertension

Central Nervous System The hypothesis that essential hypertension is due to vasoconstriction of nervous origin has always attracted great support, largely, I think, because the action of the vasomotor nerves appeals to the student as one of the few tangible facts in the circuitous behavior of the circulation Moreover, it has led to a

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the nerves leads to hypertension and tachycardia. It was supposed that in some way, as by local degenerative changes, the end organs in the carotid sinus and aortic arch ceased to be stimulated by the intravascular pressure, and evidence was produced to show that this was so We were able to show, however, that, using Hering's methods of testing, the carotid sinus reflex was still active in hypertension, though we could not say whether it was more or less so than normal (130). Similar results were obtained at the same time by Gammon (55), and for these reasons and because tachycardia is absent the hypothesis has now been abandoned Although a precise answer is not possible, the effects of compressing the common carotid artery below the sinus and the usual normal pulse rate in chronic human hypertension suggest that these reflexes have become adapted to the new and raised pressure during prolonged hypertension, that is to say, the efferent discharge of the reflex is of about the same order in chronic hypertension as in subjects with normal arterial pressure.

(2) Irritability of the vasomotor center Evidence was produced to show that the vasomotor center was rendered unduly excitable by ischemia due to vascular sclerosis It was always a little difficult to explain why the respiratory center escaped from the effect of these changes in the composition of the blood bathing the medullary cells, and this concept has now been abandoned by most workers

(3) Impulses from the higher centers Arterial pressure rises in certain circumstances associated with a changed activity of the mind, such as anxiety, fear, and mental conflict It has been supposed that the patient with essential hypertension has a disturbance of his mind such that mental situations of this type arise with unusual frequency and intensity, and that the center is kept in a state of excitation which, while highly variable, is in general much greater than normal. A similar hypothesis has been suggested for a number of diseases of so-called psychosomatic origin, which, to the clinician unobsessed with such hypotheses, seem perfectly distinct The writer doubts whether this hypothesis in its present state merits further consideration

It is extremely difficult to provide clear-cut evidence as to whether or not the abnormal constriction in essential hypertension is or is not of nervous origin In normal subjects a considerable

TABLE II

Comparison of Rates of Blood Flow Through Tissues of Normal Subject and Patient with Essential Hypertension, Each Having a Body Surface of 1.73 Square Meters

	Normal subject, cc /min	Patient with essential hypertension, cc /min.
Cardiac output (*a*)	5,900	5,900
Muscle (*b*)	800	1,360
Kidney (*c*)	1,300	800
Liver, including gut (*d*)	1,400	1,400
Brain (*e*)	810	810
Other organs (*f*)	1,590	1,530

Note The basis of these figures is as follows:

(*a*) Cardiac output. Normal values given by Lauson, Bradley, and Cournand (104) for determinations by cardiac catheter; the same figure taken for essential hypertension

(*b*) Muscle Abramson and Fierst (2) obtained the following average figures at 32 C Forearm normal, 1.77, hypertension, 2 86 cc./100 cc /min ; Calf · normal, 1 38, hypertension, 2 38 cc /100 cc./min Barcroft and Edholm (12) point out that at bath temperature of 34 C., which preserves normal values for deep muscle temperature, forearm flow averages 3 1 cc /100 cc /min The calculation has been made by correcting Abramson and Fierst's average flows by 3.1/1.77 and accepting Testut's estimate of 30 kg of muscle for a 70 kg man

(*c*) Kidney Figures from Goldring *et al* (65), and based on diodrast clearance Figure for essential hypertension is an arbitrary selection of a representative value

(*d*) Liver Figures from Culbertson *et al* (38) obtained by clearance of bromsulfalein and hepatic vein catheterization

(*e*) Brain Figures from Kety *et al* (96), using the nitrous oxide method and assuming brain weights of 1 5 kg.

(*f*) Other tissues by subtracting sum of *b*, *c*, *d*, and *e* from cardiac output.

form of therapeutics which, though far from curative, has brought amelioration to some Nerves do not overact without a reason, and we may consider briefly some of the suggestions made as to the cause of overaction

(*1*) Failure of the carotid sinus and depressor reflexes. We have seen that the end organs of the carotid sinus and depressor reflexes are constantly stimulated by the normal arterial pressure Section of

the nerves leads to hypertension and tachycardia It was supposed that in some way, as by local degenerative changes, the end organs in the carotid sinus and aortic arch ceased to be stimulated by the intravascular pressure, and evidence was produced to show that this was so. We were able to show, however, that, using Hering's methods of testing, the carotid sinus reflex was still active in hypertension, though we could not say whether it was more or less so than normal (130). Similar results were obtained at the same time by Gammon (55), and for these reasons and because tachycardia is absent the hypothesis has now been abandoned Although a precise answer is not possible, the effects of compressing the common carotid artery below the sinus and the usual normal pulse rate in chronic human hypertension suggest that these reflexes have become adapted to the new and raised pressure during prolonged hypertension, that is to say, the efferent discharge of the reflex is of about the same order in chronic hypertension as in subjects with normal arterial pressure

(2) Irritability of the vasomotor center Evidence was produced to show that the vasomotor center was rendered unduly excitable by ischemia due to vascular sclerosis It was always a little difficult to explain why the respiratory center escaped from the effect of these changes in the composition of the blood bathing the medullary cells, and this concept has now been abandoned by most workers.

(3) Impulses from the higher centers Arterial pressure rises in certain circumstances associated with a changed activity of the mind, such as anxiety, fear, and mental conflict It has been supposed that the patient with essential hypertension has a disturbance of his mind such that mental situations of this type arise with unusual frequency and intensity, and that the center is kept in a state of excitation which, while highly variable, is in general much greater than normal. A similar hypothesis has been suggested for a number of diseases of so-called psychosomatic origin, which, to the clinician unobsessed with such hypotheses, seem perfectly distinct The writer doubts whether this hypothesis in its present state merits further consideration.

It is extremely difficult to provide clear-cut evidence as to whether or not the abnormal constriction in essential hypertension is or is not of nervous origin In normal subjects a considerable

fraction of vascular tone is due to the action of the vasomotor nerves, and this fraction might persist in hypertension even though the abnormal constriction were of non-nervous origin. It is thus not enough to show that paralysis of the vasomotor nerves produces vasodilatation in hypertension. To prove the nervous hypothesis it must be shown that the vessels are reduced to the same state in the subject with hypertension as they would be in a normal subject in similar circumstances Observations involving the circulation as a whole, particularly when the parts played respectively by cardiac output and vasomotor changes are unknown, are very difficult to interpret accurately. These include such effects as those of narcosis, of spinal anesthesia, and of tetraethylammonium bromide. Experiments involving a part of the circulation have given a clear-cut answer Thus, in the hand, vascular narrowing persists when the influence of the vasomotor nerves is removed (123). In the calf, Wilkins and Eichna's observations, though not systematically directed to this question, also revealed flows that were not abnormally large after sympathectomy (162). In the kidney, efferent arteriolar constriction persists after sympathectomy (64, 65). These results, obtained on three different and important vascular territories, and entirely consistent one with another, make it extremely difficult to believe that in essential hypertension the fault lies in the action of the vasomotor nerves Vasoconstriction of nervous origin there is, but there is no unequivocal evidence that this is abnormal in degree

Hypothesis of Renal Ischemia It was the old observation, subsequently confirmed repeatedly, of the frequency with which organic vascular narrowing is found in the kidneys of patients dying of essential hypertension, that led Goldblatt to try the effect of clamping the renal artery The success thereby achieved has led him and many others to believe that the hypothesis will in the long run prove to be the correct one. This hypothesis holds that the primary change in essential hypertension is arteriolosclerosis in the kidney occasionally obstruction to the main renal artery In favor of this hypothesis are the following facts·

(1) The experimental hypertension resembles essential hypertension in many of its main features, and is the only form of experimental hypertension that does so

(2) Moritz and Oldt's (116a) observations, showing that the in-

cidence of arteriolosclerosis follows the same order of intensity in a variety of organs in subjects with normal and raised pressures, except for the kidney, which is nearly always involved in subjects with raised pressures, rarely involved in those with normal pressures (Fig 5).

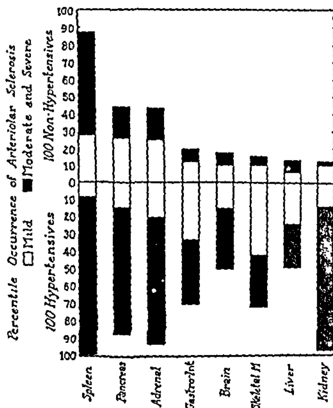


Fig 5 Comparison of occurence of arteriolar sclerosis in various organs and tissues of 100 subjects with hypertension and 100 subjects with normal blood pressure (116a)

(3) The absence of any proved difference in the pattern of the circulation in essential hypertension and in those with unilateral pyelonephritis, in which excising the kidney abolishes hypertension

(4) The evidence, already detailed, suggesting that in essential hypertension the agent is humoral and not nervous

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ably not the only change leading to hypertension, and that particularly in chronic hypertension it is inadequate to account for all the phenomena involved. The same position is found in essential hypertension. While the earlier experiments of Prinzmetal and Friedman (135) had suggested that the renin content of the kidney was raised in essential hypertension, more exact estimations by Landis (101) have failed to substantiate this Haynes *et al.* (79a) have obtained renal vein blood by catheter from patients with essential hypertension and subjects with normal pressures, and with the indirect method of assay have been unable to detect significant amounts of renin. But perhaps the most severe blow to the renin hypothesis has been the finding by Merrill *et al.* (116) of significant amounts of renin in the renal vein blood of 8 out of 11 cases of congestive failure. For in cardiac failure recent observations have also indicated the presence of efferent arteriolar vasoconstriction If, in fact, renin is released into the renal vein in cardiac failure in amounts detectable by present methods without producing hypertension, then it is difficult to believe that the release of renin can be a significant factor in producing raised arterial pressure in a condition such as essential hypertension, when the renin content in renal vein blood is too small to detect.

The reader is now in a position to judge of the importance of a precise answer to the question as to what raises the arterial pressure in chronic hypertension from renal artery constriction For until this answer is known, it may prove impossible to answer the question whether raised arterial pressure in essential hypertension is or is not primarily due to a renal abnormality.

Other Possible Mechanisms

No good purpose can be served by a long discourse, for there is no other hypothesis that is precisely enough formulated or adequately supported by evidence to justify discussion But it may be justifiable to recall one point, as yet imperfectly established, namely, the probability that essential hypertension is an inherited condition Is it possible, for example, that the primary abnormality is a metabolic disturbance affecting the muscular elements of the arterial and arteriolar walls, so that these contract more strongly in response to those chemical and nervous factors that are responsible for nor-

Against the hypothesis are cited the following: (*1*) The observation by Castleman and Smithwick (28) that in biopsies of kidneys taken at operation for sympathectomy in patients with early essential hypertension, renal arteriolar lesions were entirely absent in 8 per cent and slight or moderate in about 40 per cent of cases (*2*) The observations of Goldring *et al* (65), showing that the dominant feature of the renal circulation is efferent arteriolar constriction, probably of humoral origin, and expressive of the abnormal agent responsible for hypertension, and the absence in early cases of other evidence of renal ischemia

In judging these rival arguments it is essential that we rid our minds in the first place of the renin hypothesis which will be discussed presently If this is done, then the writer agrees with Goldblatt (60) in the view that the hypothesis of renal ischemia is still not disproved. For although we can not be certain of the exact change which is necessary to excite the renal mechanism, we do know that it operates locally within the kidney and independently of its nervous connections, and that it is stimulated by constricting the renal artery Now, the kidney may be looked on as series of nephrons, and it is possible that focal arteriolar sclerosis may stimulate the local intrarenal mechanism in those nephrons it affects, though not in the remainder. It is conceivable, therefore, that there may be enough nephrons affected in this way to produce hypertension, though the over-all picture of the circulation is dominated by the effect of the humoral effector agent on the remainder Thus, the evidence of Goldring and his colleagues (65) does not disprove the renal ischemia hypothesis, though at the same time it offers none in its favor Again, Goldblatt has pointed out that the small pieces of kidney examined by Castleman and Smithwick do not prove the absence of arteriolar lesions in the remainder of the kidney. The verdict given on the evidence is thus an open one, the hypothesis is found *neither proved nor disproved*

As we have seen, there seemed at one time a strong possibility that renal hypertension was due to the release of abnormal quantities of renin into the blood stream, neither more nor less than this The mechanism of hypertension due to renal artery constriction thus became identified in men's minds with the renin hypothesis We have seen that the release of renin from the kidney is prob-

ably not the only change leading to hypertension, and that particularly in chronic hypertension it is inadequate to account for all the phenomena involved. The same position is found in essential hypertension While the earlier experiments of Prinzmetal and Friedman (135) had suggested that the renin content of the kidney was raised in essential hypertension, more exact estimations by Landis (101) have failed to substantiate this. Haynes *et al* (79a) have obtained renal vein blood by catheter from patients with essential hypertension and subjects with normal pressures, and with the indirect method of assay have been unable to detect significant amounts of renin But perhaps the most severe blow to the renin hypothesis has been the finding by Merrill *et al.* (116) of significant amounts of renin in the renal vein blood of 8 out of 11 cases of congestive failure For in cardiac failure recent observations have also indicated the presence of efferent arteriolar vasoconstriction If, in fact, renin is released into the renal vein in cardiac failure in amounts detectable by present methods without producing hypertension, then it is difficult to believe that the release of renin can be a significant factor in producing raised arterial pressure in a condition such as essential hypertension, when the renin content in renal vein blood is too small to detect.

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mal vascular tone? No evidence that is worth discussing exists for or against this hypothesis at the present time.

ACUTE NEPHRITIS

It is very much easier to identify those changes in the circulation that accompany hypertension in acute nephritis, for in the vast majority of cases, the hypertension is transient. Repetition of the observations when the phase of hypertension is terminated, and particularly when acute nephritis is cured, provides normal figures that are more directly comparable with the abnormal than those that are won from other persons with peculiarities of their own. It is therefore rather surprising to find how few data there are: no doubt, because the hypertension is transient, and does not always coincide with opportunity.

The cardiac output was found by Hayasaka (78) slightly higher in one case of acute nephritis during the phase of hypertension than after its cure; he used the method of triple extrapolation. The writer observed no significant change in circulation time (123). These methods are open to criticism, and estimations by the Fick principle using the cardiac catheter are urgently needed. The hemoglobin and red cells are usually reduced in the hypertensive phase, and blood viscosity is lowered (123). If, in fact, the cardiac output proves normal, the hypertension will have been shown to be due to vasoconstriction.

Estimations of blood flow through the hand in 6 cases of acute nephritis (123) showed that, when vasomotor nervous tone had been released, significantly higher flows were obtained during the phase of hypertension than after it in 4 patients; in the remaining 2 there was no change, but in these the flows were high in the absence of hypertension The relationship of hand blood flow to diastolic pressure in these 4 cases of acute nephritis was of the same order as was found in a case of femoral arteriovenous fistula, in which the diastolic pressure could be raised by digital obliteration of the artery proximal to the fistula and in which the hand vessels were also freed of vasomotor nervous tone In contrast, therefore, to essential hypertension and to chronic nephritis, no evidence was found in these cases of acute nephritis of a non-nervous constriction of the hand vessels These observations have been, in the main, confirmed by Arnott and Matthew (9)

Fouts, Corcoran, and Page (49) determined inulin and phenol red clearances in dogs with nephrotoxic nephritis produced by injection of anti-dog kidney serum by the method of Masugi They observed a greater fall in inulin than in phenol red clearance, and attributed this discrepancy to swelling of the glomerular basement membrane. Hypertension did not develop. Black and co-workers (17) determined inulin and diodrast clearances in 3 patients with acute nephritis and hypertension, and in 2 of them after nephritis had subsided and the hypertension had gone. They found a greatly reduced inulin clearance, a moderately reduced diodrast clearance, and an abnormally low filtration fraction in all 3 cases. In the 2 cases, the filtration fraction returned to normal after the hypertension had subsided.

Now it is to be remarked that the interpretation of inulin and diodrast clearances in nephritis, where the anatomic disturbance of the kidney is great, is fraught with difficulty, and we find the low filtration fraction differently explained by different workers; Fouts and others attributed it to swelling of the glomerular basement membrane, while Black and colleagues attribute it to constriction of the afferent glomerular arteriole Nevertheless, the renal studies clearly do not reveal evidence of efferent arteriolar constriction, such as characterizes the kidney in essential hypertension and is found during the response to renin and hypertensin Taken in conjunction with the studies in hand blood flow which failed to reveal evidence of vasoconstriction of humoral origin, it is clear that acute nephritis is one of the forms of human hypertension which is unlikely to be due to the action of renin Yet the presence of renin in the systemic blood is reported in 2 cases of acute nephritis and hypertension by Braun Menendez and others (26) and in 1 case by Dexter and Haynes (40) Clearly, here is a major discrepancy in the evidence which future work must attempt to elucidate

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and thus the ratios of glomerular filtration rate to minimal effective renal blood flow and to tubular excretory mass are diminished This is interpreted as being due largely to a distortion of the normal ratio of glomerular and tubular function occasioned by the disease Hypertension tends to raise the abnormally low filtration fraction to or beyond the normal limits. The disturbance in excretion of these substances in chronic nephritis is clearly a product of both the functional and the circulatory disturbances in the kidney that accompany the disease, and the picture that emerges of the renal circulatory abnormality associated with hypertension is far from clear. There are no figures available for the blood flow through other tissues of the body.

From time to time it has been stated that the behavior of hypertension shows clear-cut differences in essential hypertension and chronic nephritis Many of these alleged differences have been unconfirmed on repetition or have been explicable on the basis of age difference (122). Dexter and Haynes (40) state that they have been unable to detect renin in the systemic blood of patients with chronic nephritis, even with arterial pressures as high as 285/180

The circulatory abnormalities accompanying hypertension in chronic nephritis are thus very imperfectly understood The only clear implication is that obtained from a study of the hand circulation, namely, of vasoconstriction that is not of vasomotor nervous origin

PYELONEPHRITIS

Raised arterial pressure occurs only in some cases of pyelonephritis. For a discussion of the factors in the renal lesion which determine the presence or absence of hypertension, the reader is referred to other articles (64, 156). Here we are chiefly concerned with those cases in which the disease appears to be unilateral, in which intravenous pyelography reveals grossly diminished or absent excretion on one side, and in which retrograde pyelography reveals an abnormality of pelvis and calyces on the same side, usually a hydronephrosis When such a kidney is excised it is found to be small, to have a hydronephrosis with gross reduction of renal substance, and showing much organic arterial thickening and pyelonephritic infiltration of cells Published reports give an unreal impression of the effects on hypertension of excising such kidneys,

occurs during the acute phase of this nephritis, but according to Arnott and Kellar it fails to occur after renal denervation (8). No increase in the renin content of kidneys of rabbits with this form of nephritis was found by Arnott and Kellar in conjunction with the writer and Prinzmetal. But in acute nephritis in man, the hypothesis that hypertension is due to a renal disturbance must take cognizance of one major objection, namely, that the onset of *hypertension precedes the onset of albuminuria* In 1917, Nonnenbruch (117) showed that in many cases of the acute nephritis of wartime, edema and hypertension preceded by several days the appearance of blood and albumen in the urine. The temporal precedence of hypertension over albuminuria and hematuria has been confirmed repeatedly for the acute nephritis following scarlatina and other acute infections by Kylin (100), Koch (97) and Bayart (13). For this reason, and because the edema fluid has been found to contain more protein than in some other conditions, many workers have considered *that acute nephritis is a very generalized vascular disturbance* of which the renal lesion is but an expression rather than the cause.

CHRONIC NEPHRITIS

So far as we know, cardiac output in chronic nephritis is not raised unless severe anemia develops, as it does in the terminal stages of uremia Blood viscosity is normal or decreased Hypertension is therefore due to peripheral vascular narrowing The evidence suggesting that this is functional rather than organic is of the same kind as already discussed under essential hypertension

Calorimetric estimations of hand blood flow after release of vasomotor nervous tone revealed a precisely similar situation to that already described for essential hypertension, namely a persistence of abnormal vascular narrowing (122) In one case of progressive chronic nephritis, a rise of arterial pressure was found to be accompanied by a slight fall in the heat elimination from the hand (124).

The excretion of inulin and diodrast by the kidney in the various stages of chronic nephritis, with and without hypertension, have been studied by Earle, Taggart, and Shannon (43) These studies reveal a depression of inulin clearance, diodrast clearance, and the maximal rate of tubular excretion of diodrast as the disease advanced. In general, the glomerular filtration rate is the most affected,

and thus the ratios of glomerular filtration rate to minimal effective renal blood flow and to tubular excretory mass are diminished This is interpreted as being due largely to a distortion of the normal ratio of glomerular and tubular function occasioned by the disease Hypertension tends to raise the abnormally low filtration fraction to or beyond the normal limits. The disturbance in excretion of these substances in chronic nephritis is clearly a product of both the functional and the circulatory disturbances in the kidney that accompany the disease, and the picture that emerges of the renal circulatory abnormality associated with hypertension is far from clear. There are no figures available for the blood flow through other tissues of the body.

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as those cases in which hypertension is abolished tend to be published, the others not My personal experience is of 4 such cases In 2, nephrectomy had no effect on the hypertension, in 1 of these autopsy revealed pyelonephritic changes in the other kidney; in the other, persistent albuminuria suggested that the other kidney was in fact not normal In 1 in whom ligature of an aberrant renal artery was followed by hypertension, excision of the kidney 6 years ago has led to persistently normal arterial pressure; and in 1 with malignant hypertension, excision of the kidney 2 years ago has led to a significant fall in arterial pressure with complete resolution of albuminuric retinitis. Of 11 cases reported by Langley and Platt (103a) there was 1 complete success, 1 partial success, and 9 failures; but in most of the failures there was, as in my experience, evidence that the disease was in fact bilateral. These 11 cases contained examples of apparent unilateral renal disease other than the atrophic pyelonephritis before mentioned

These cases are of the greatest importance in the study of hypertension, because those in which nephrectomy abolishes hypertension completely and permanently provide the only unequivocal evidence that the hypertension is due to a renal cause In the case of malignant hypertension mentioned above, Scarborough found that the blood flow through the hand from which vasomotor nervous tone had been removed was essentially unaltered by the very conspicuous fall in arterial pressure. The suggestion of a humoral vasoconstriction in the hand therefore arises in such cases.

PREGNANCY KIDNEY AND ECLAMPSIA

Here again is an acute hypertension that is transient, the arterial pressure in most instances falling to normal after parturition But again we are still relatively ignorant of the cause of the hypertension In a brief summary of work done before the last war Kellar and Sutherland (95) state that calorimetric estimates of hand blood flow after elimination of vasomotor nervous tone were normal in 35 cases of toxemia, but give no details The renal circulation has been investigated by Corcoran and Page (36), Chesley and others (32) and Wellen and others (158), all of whom are in substantial agreement The glomerular filtration rate and the tubular excretory mass tend to be normal, while the effective renal blood flow is in-

creased It is suggested that these changes are due to efferent glomerular dilatation, with possible thickening of the glomerular basement membrane. Here, then, is yet another pattern of the circulation in hypertension, but its cause remains obscure. Curiously enough, there are again reports of abnormal amounts of renin in systemic blood (40), and since these assays were made by the incubation method they can not very well refer to any other pressor substance. The pattern of the renal circulation in eclampsia is quite unlike that said to characterize the action of renin.

COARCTATION OF THE AORTA

Lewis (108) drew attention to the frequency of hypertension in the upper limbs in cases of coarctation of the aorta surviving to adult life. Estimations of arterial pressure in the lower limbs by the auscultatory method gave lower and often normal figures. Lewis found blood flows through the upper and lower limbs that were essentially normal in coarctation, but these should be repeated, since he did not occlude hand and foot. The conclusion, therefore, arrived at was of an abnormally high peripheral resistance in the upper part of the body only. Subsequent measurements of arterial pressure by direct arterial puncture and high frequency manometer by Steele (147) showed that while the systolic pressure was much higher in the upper limbs, the diastolic pressure was raised to the same extent both above and below the coarctation It therefore seems that vascular narrowing in coarctation is generalized Prinzmetal and Wilson (135a) found increased blood flows through the upper limb after releasing nervous tone by warming the body, but they used the plethysmographic method before the importance of differentiating between hand and forearm flow was known Calorimetric estimations of blood flow through the hand after releasing vasomotor nervous tone yielded essentially normal values in 3 cases of coarctation (122) At the time these measurements were made, the rise of diastolic pressure below the constriction was unknown, and a humoral mechanism was therefore not contemplated But since Steele's demonstration, it is clear that these results are most consistent with a vasoconstriction of humoral origin, as in essential hypertension and chronic nephritis

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as those cases in which hypertension is abolished tend to be published, the others not. My personal experience is of 4 such cases In 2, nephrectomy had no effect on the hypertension; in 1 of these autopsy revealed pyelonephritic changes in the other kidney; in the other, persistent albuminuria suggested that the other kidney was in fact not normal. In 1 in whom ligature of an aberrant renal artery was followed by hypertension, excision of the kidney 6 years ago has led to persistently normal arterial pressure; and in 1 with malignant hypertension, excision of the kidney 2 years ago has led to a significant fall in arterial pressure with complete resolution of albuminuric retinitis Of 11 cases reported by Langley and Platt (103a) there was 1 complete success, 1 partial success, and 9 failures; but in most of the failures there was, as in my experience, evidence that the disease was in fact bilateral. These 11 cases contained examples of apparent unilateral renal disease other than the atrophic pyelonephritis before mentioned.

These cases are of the greatest importance in the study of hypertension, because those in which nephrectomy abolishes hypertension completely and permanently provide the only unequivocal evidence that the hypertension is due to a renal cause In the case of malignant hypertension mentioned above, Scarborough found that the blood flow through the hand from which vasomotor nervous tone had been removed was essentially unaltered by the very conspicuous fall in arterial pressure The suggestion of a humoral vasoconstriction in the hand therefore arises in such cases.

PREGNANCY KIDNEY AND ECLAMPSIA

Here again is an acute hypertension that is transient, the arterial pressure in most instances falling to normal after parturition But again we are still relatively ignorant of the cause of the hypertension In a brief summary of work done before the last war Kellar and Sutherland (95) state that calorimetric estimates of hand blood flow after elimination of vasomotor nervous tone were normal in 35 cases of toxemia, but give no details The renal circulation has been investigated by Corcoran and Page (36), Chesley and others (32) and Wellen and others (158), all of whom are in substantial agreement The glomerular filtration rate and the tubular excretory mass tend to be normal, while the effective renal blood flow is in-

creased. It is suggested that these changes are due to efferent glomerular dilatation, with possible thickening of the glomerular basement membrane Here, then, is yet another pattern of the circulation in hypertension, but its cause remains obscure. Curiously enough, there are again reports of abnormal amounts of renin in systemic blood (40), and since these assays were made by the incubation method they can not very well refer to any other pressor substance The pattern of the renal circulation in eclampsia is quite unlike that said to characterize the action of renin.

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origin of the renal arteries yields hypertension while clamping below does not (61), it has been supposed that the hypertension of coarctation of the aorta is due to renal ischemia (139). This may be the explanation, but the writer does not clearly see why, if the arterial pressure is in fact elevated below the coarctation, the kidneys should receive less blood than normal. Nor if reduced intrarenal arterial pressure is the cause of hypertension is the defect apparent in coarctation If the stimulus is reduced pulse pressure, as Page postulates, then the mechanism holds Friedman and others (54) have measured the inulin and diodrast clearances in 6 cases of coarctation with hypertension and have inferred normal glomerular filtration rates with moderate reductions in renal blood flow and a high filtration fraction. They interpret these results as indicating a primary reduction in renal blood flow with a secondary spasm of the efferent glomerular arteries; but it is not clear to the writer why the whole picture can not be explained on constriction of the efferent arterioles; nor on what evidence they conclude that there is a primary reduction in renal blood flow.

The recent demonstration that the aortic defect can be repaired surgically, with consequent return of the arterial pressure to normal, offers an exceptional opportunity for further study of this interesting condition

Conclusion

The reader will now appreciate that much remains to be done before we have a clear understanding of hypertension. Experiments on animals will no doubt acquaint us further with the mechanisms responsible for the control of vascular tone. From the clinical point of view, it is imperative that patient collection of accurate information concerning the state of the circulation and its changes in response to events which terminate hypertension should go on, for it is only when we have enough evidence to work on that we shall be able to put our finger on the key to the puzzle

Addendum

Between the stages of manuscript and proof new work requires some modification in detail of views expressed in the foregoing account. Biologic assay has revealed that the chief substance released

by stimulating the splenic sympathetic nerves in the cat is noradrenaline (119a). Noradrenaline is also the chief constituent of phaeochromocytoma (91a). Noradrenaline in man produces gross constriction of the skin vessels, constriction of muscle vessels, pallor of the gut, efferent arteriolar constriction of the kidney, a rise in both systolic and diastolic pressure and bradycardia of vagal origin (12a–b, 63a, 147a). Noradrenaline is probably not the agent concerned in human essential hypertension, because: (*1*) in phaeochromocytoma even between the attacks the maximum heat elimination of the hand is reduced below the normal level to which it returns after removal of the tumour (12b); and (*2*) noradrenaline pales the facial skin in essential hypertension when injected intravenously in doses with a small effect on blood pressure (12b).

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